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25° ESCV 2023

30 AUGUST – 2 SEPTEMBER

ABSTRACT BOOK

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ORAL PRESENTATIONS

OP 001

Advancements in diagnostics

DETERMINANTS OF POSITIVITY AND IMPACT OF CLINICAL METAGENOMICS: A 3 YEARS' PROSPECTIVE STUDY IN FRANCE

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BACKGROUND-AIM

Metagenomic next-generation sequencing (mNGS) allows untargeted identification of a broad range of pathogens, including rare or novel microorganisms. We aimed to assess the determinants of positivity and clinical pertinence of mNGS.

METHODS

We prospectively performed short-read shotgun metagenomics analysis as a second-line technique or as a first-line test in life-threatening situations. We describe variables associated with the detection of "causative" (i.e., known to cause similar clinical presentations) or "probable causative" (i.e., known to be associated with similar clinical presentations without formal evidence of causality) pathogens by mNGS.

RESULTS

We analyzed 743 specimens collected from 523 patients between November 2019 and November 2022. Microorganisms were detected in 41% of the samples. Causative/probably causative pathogens were detected in 25% of samples from patients with initial suspicion of infection, versus 3% of samples analyzed to rule infection out (OR=9·1, p<0·001). We showed that mNGS on cerebral biopsies had higher odds of detecting a causative pathogen than mNGS on CSF (OR=4·1, p=0·002) and that compared to immunocompetent individuals, causative/probably causative pathogens were more frequently identified in samples from immunodeficient patients (25% versus 9%, respectively; OR=2·4, p=0·001).
Concordance with conventional confirmatory tests results was 95% when mNGS detected causative/probably causative pathogens.

CONCLUSIONS

Results of this study show the potential benefit of adding mNGS to routine diagnosis workflows both for detection and discovery of rare or novel pathogens. We identified major determinants of clinical benefits especially in immunocompromised patients and in subjects with available brain biopsies and/or stool samples. mNGS seems to have not only the highest sensitivity in cases where the primary diagnostic hypothesis is an infection, but also a good negative predictive value in subjects in whom clinicians aim to exclude the presence of a pathogen.

OP 002

Advancements in diagnostics

PERFORMANCE OF METAGENOMICS NGS PROBE-BASED PATHOGEN DETECTION IN CLINICAL SAMPLES

R. Ferreira [1], L. Coelho [1], D. Sobral [1], J. Dourado [1], J. Isidro [1], V. Mixão [1], M. Pinto [1], A. Nunes [1], S. Duarte [2], L. Vieira [2], V. Borges [1], J.P. Gomes [1]

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BACKGROUND-AIM

In a clinical setting, metagenomic Next-Generation Sequencing (mNGS) may rapidly and accurately detect unsuspected, unidentified and uncultivatable organisms, resulting in higher recovery outcomes due to timely and more specific treatments. However, mNGS presents several challenges, such as: high costs per sample; a tremendous small microbial/human DNA ratio; the need for a multidisciplinary team; and demanding laboratorial and computational infrastructures.

METHODS

We tested two recently developed Illumina panels for probe-based pathogen enrichment, the Respiratory and the Urinary Pathogen ID/AMR panels (RPIP and UPIP), which together target 383 pathogenic agents (70 virus, 247 bacteria, 62 fungi and 4 parasites). We selected a total of 81 clinical samples of different nature (e.g., CSF, plasma, serum, urine, swabs, biopsies, etc.), for which at least one pathogen had been identified by PCR. Data analysis was performed using both Illumina and in house bioinformatics pipelines, with panels' performance being assessed in a combined fashion to mimic a real scenario where the panels would be simultaneously used to cover all 383 pathogens.

RESULTS

Herein, 77% of the PCR-confirmed pathogens (virus, bacteria, fungi and parasites) were detected by the Illumina toolkit (probe-design and pipeline). Focusing on clinical virology, we also assessed whether the viral detection rate could be further improved using a recently developed bioinformatics pipeline for virus metagenomics detection (INSaFLU-TELEVIR, https://insaflu.insa.pt/). With this complementary approach, the initial Illumina accuracy for virus detection increased from 74.1% to 84.5%. As such, we are currently developing a TELEVIR-like pipeline for bacteria to assess if a similar increment in pathogen detection is also achieved. Finally, we observed some heterogeneity in the pathogen identification accuracy among different sample types, ranging from 67% in CSF to 100% in plasma.

CONCLUSIONS

Although these results rely on preliminary data, they provide strong evidence that these Illumina panels (preferentially coupled with complementary downstream pipelines) may constitute a powerful tool to help difficult diagnosis and support clinical decision, in which timely and specific treatment may be decisive for patients' clinical recovery.

OP 003

Advancements in diagnostics

RAPID AND SENSITIVE SINGLE-SAMPLE VIRAL METAGENOMICS USING NANOPORE FLONGLE SEQUENCING

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BACKGROUND-AIM

The ability of viral metagenomic Next-Generation Sequencing (mNGS) to unbiasedly detect nucleic acids in a clinical sample is a powerful tool for advanced diagnosis of viral infections. When clinical symptoms do not provide a clear differential diagnosis, extensive laboratory testing with virus-specific PCR and serology can be replaced by a single viral mNGS analysis. However, widespread diagnostic use of viral mNGS is thus far limited by long sample-to-result times, as most protocols rely on Illumina sequencing, which provides high and accurate sequencing output but is time-consuming and expensive.

METHODS

We developed a novel mNGS protocol based on the cost-effective Nanopore Flongle sequencing with decreased sample preparation time of six hours followed by approximately 2 hours real-time sequencing. Separate detection of DNA and RNA viruses is achieved by two workflows for anchored amplification using random primers, DNase treatment and reverse transcription (only RNA workflow), second strand synthesis, and subsequent barcoding PCR. The two workflows are then pooled before sequencing on a single Flongle. Reads were analyzed in real-time using Fastq WIMP (Human and Viral) by EPI2ME.

RESULTS

The novel method was validated on spiked samples and diverse clinical isolates. The total turnaround time of eight hours is substantially reduced compared to Illumina mNGS. Although Flongles yield lower sequencing output, direct comparison with Illumina mNGS showed high agreement (PPA = 91.67 %, PPV = 100 %). Both DNA and RNA viruses were still detected at a low input of 35 to 40 cycle thresholds of specific (RT-)qPCR.

CONCLUSIONS

The novel Nanopore mNGS approach is specifically tailored for use in clinical diagnostics and provides a rapid, cost-effective, and sensitive viral mNGS strategy for individual testing of severe cases where a fast diagnosis is crucial.

OP 004

Advancements in diagnostics

THE NEXT GENERATION OF CLINICAL VIROLOGY

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BACKGROUND-AIM

The application of next-generation sequencing in clinical virology is gathering momentum given its potential for unbiased pathogen detection, in addition to comprehensive genetic characterization and monitoring of viral infections. However, current challenges include the standardization of protocols, and most importantly the analysis and interpretation of data. Regarding the latter, much is yet to be learned on the virome of healthy individuals to evaluate the significance of viral findings in various disease states. In a recent study by us (Pyöriä et al., *Nucleic Acids Research*, 51:7,3223–3239) we showed that many more viruses than ever imagined reside lifelong in the human body.

METHODS

By integration of quantitative (qPCR) and qualitative (hybrid-capture sequencing) analysis, we studied the prevalence and distribution of human eukaryotic DNA viruses in 9 organs (colon, liver, lung, heart, brain, kidney, skin, blood, hair) of 31 recently deceased Finnish individuals.

RESULTS

We identified the DNAs of 17 species, primarily herpes-, parvo-, papilloma- and, anello-viruses (>80% prevalence), typically persisting in low copies (mean 540 copies/ million cells). The highest viral diversity was detected in the colon, liver, and kidney. Moreover, we provided the highest resolution to date of the genetic makeup of the human virome, reporting over 70 high-quality viral genomes. We show that persistent viruses share high sequence homology across organs.

CONCLUSION

Our findings reveal unprecedented prevalences of viral DNAs in human organs and provide a fundamental ground for the interpretation of NGS-based diagnostics, as well as for the investigation of disease correlates. Our study highlights the need for a comprehensive understanding of the prevalences, quantities, and genomic diversity of the virome for discrimination of deviations in various disease states.

OP 005

Clinical cases

ZIKA VIRUS INFECTION IN TWO FAMILY MEMBERS RETURNING FROM MALDIVES

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BACKGROUND-AIM

Zika virus (ZIKV) is a Flavivirus, causing outbreaks in Africa, in the Pacific and South America. ZIKV is associated to Guillan-Barré syndrome and fetal growth alterations. Two viral lineages were identified, namely African and Asian. After the large 2015-2016 Latin America outbreak, the virus is sporadically detected outside endemic regions. Here we describe ZIKV infection in family members returning from Maldives.

METHODS

At the presentation at Niguarda Emergency Room (ER), anamnesthic, clinical and epidemiological information were recorded. Saliva, urine and serum specimens were collected for molecular and serological tests: RT-PCR (quanty ZIKA, Clonit Srl, Italy) was performed on all samples, confirming results with a home-made PCR and Sanger sequencing (Patel et al, 2013); IgM and IgG were assayed on serum (ZIKA VIRCLIA® IgG and IgM MONOTEST, VIRCELL S.L., Spain; positive cut-off > 1.1 COI).

RESULTS

On May 2nd, 2023, a 38 years-old woman presented at ER with fever, headache, myalgia, laterocervical lymphadenopathy, rash and distal edema. She disclosed a travel to Maldives from 20 to 28 April, with her husband and their three children: the man had fever and myalgies without rash; children were asympthomatic. Diagnostic tests were performed on parental samples. The RealTime-PCR returned positive results on all specimens: the highest concentrations were in paternal (10^6 copies/mL) and maternal (10^5 copies/mL) urine, used for home-made PCR and Sanger. Amplification was detected on agarose gel; the 250 bp fragment sequences was submitted to BLAST, that aligned them to ZIKV records included in the Asian lineage, as confirmed by phylogenetic analysis. Serological analysis showed the presence of IgM in both adults sera (3.97 and 3.72 COI), while IgG were present only in the woman one (4.69 COI).

CONCLUSIONS

This is the first ZIKV Italian case since the last October 2022 official report. Such occurrence stress the importance of surveillance in returning travellers, especially considering the re-start of journeys in endemic areas after pandemic related stop.

OP 006

Clinical cases

ACUTE RENAL FAILURE: UNCOMMON COMPLICATION OF EPSTEIN-BARR VIRUS INFECTIOUS MONONUCLEOSIS

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BACKGROUND-AIM

Epstein-Barr virus (EBV) belongs to the Herpesviridae family. If most infections are asymptomatic, primary EBV infection can present as infectious mononucleosis and be complicated by acute renal failure, mainly in immunocompromised individuals. In some rare cases, it can happen in immunocompetent patients.

METHODS

Here we report the case of a 28 years old man that came to the emergency room suffering of headache, vomiting, cervicalgia, loss of appetite, and fever. He has reported any risk of exposure to an infectious disease, however he lives with his wife and young children aged 9 months and 2 years. First diagnosis was influenza-like illness and the patient went home with a prescription for further viral respiratory testing.

RESULTS

No respiratory virus infection was detected. The patient required to be hospitalized the following day in the Nephrology department due to worsening symptoms with the onset of acute renal failure. The neutrophil count went from normal to 0.18 G/L in 4 days suggesting agranulocytosis which motivated a bone marrow aspiration. Complete blood count showed 68% of lymphocytes among which numerous hyperbasophilic cells. Bone marrow cytology did not show any alteration but viral PCR was significantly positive for EBV (175,400 IU/mL) and weakly positive for parvovirus B19 (B19V) and human herpesvirus 6 (HHV-6). EBV was also significantly detected in the blood (26,800 IU/mL). Kidney biopsy revealed interstitial edema with an inflammatory infiltrate mainly composed of lymphocytes. Immunofluorescence showed no immune deposits. Whereas immunostaining for EBV latent membrane protein 1 (LMP1) was negative, EBV-encoded RNA (EBER) in situ hybridization showed few positive interstitial inflammatory cells. Serological investigations revealed positive viral capsid antigen (VCA) IgM antibodies and negative VCA and Epstein-Barr Nuclear Antigen (EBNA) IgG antibodies indicating primary EBV infection. Moreover, the patient has been proven to be HIV-seronegative.

CONCLUSIONS

Finally, a diagnosis of acute interstitial nephritis secondary to primary EBV infection was retained. The patient recovered quickly with corticosteroid treatment and only two sessions of dialysis. One month later, renal function was fully restored.

OP 007

Clinical cases

DONOR DERIVED HERPES SIMPLEX VIRUS (HSV) HEPATITIS IN KIDNEY TRANSPLANT RECIPIENTS: A SERIES OF TWO CASES AND REVIEW OF THE LITERATURE

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BACKGROUND-AIM

Donor derived infections are less frequent but can lead to clinically important syndromes in the post-transplant setting which can include hepatitis. Here we report two renal transplant recipients confirmed to have donor derived HSV-2 infection with associated hepatitis.

METHODS

Case 1: A 42-year-old lady who received her 3rd renal transplant developed fever and abdominal pain on day 6 after transplant. Abdominal CT was suggestive of hepatitis. HSV-2 PCR was positive on whole blood. She developed severe hepatitis, leukopenia, thrombocytopenia and an AKI requiring intensive care. IV acyclovir was started and immunosuppression reduced. Her HSV2 PCR remained positive until day 61. She completed 4 weeks of iv acyclovir and 8 weeks of oral valganciclovir due to CMV reactivation She is still on on prophylactic dose acyclovir 12 months post-transplant. Her pre-transplant HSV serology was negative. Repeat serology done 4 months post-transplant showed detectable HSV-2 IgG. The donor was found to be HSV 1&2 IgG negative but had a detectable HSV-2 PCR on whole blood taken at the time of organ harvesting.

RESULTS

Case 2: A 28-year-old man who received a simultaneous pancreas and kidney transplant from the same donor developed a fever on day 8 post-transplant. He was immediately started on iv acyclovir and a whole blood PCR confirmed HSV-2. He developed a hepatitis but was otherwise well. He remained PCR positive until day 13 but then had serially negative PCRs. He completed 2 weeks of iv acyclovir, 10 weeks of oral valacyclovir and 6 months of acyclovir prophylaxis. His pre-transplant serum showed no HSV-1 or 2 IgG. He sero-converted with detectable HSV-2 IgG 9 months post-transplant after which prophylaxis was stopped. He made a full recovery with a functional kidney and pancreas.

CONCLUSIONS

Donor-derived HSV hepatitis in SOT recipients is rare but has high mortality. Diagnosis is challenging due to the non-specific presentation. The American Society of Transplantation recommends HSV-specific prophylaxis for all seropositive organ recipients who are not receiving CMV prophylaxis, given that ganciclovir and valganciclovir have anti-HSV action [2]. Our patients as well as the donor were sero-negative for CMV and hence they were not on prophylaxis which contributed to developing HSV-2 viraemia.

OP 008

Clinical cases

INVESTIGATING MULTIPLE YEARS OF WITHIN HOST EVOLUTION OF HEPATITIS E VIRUS IN A IMMUNOCOMPROMISED PATIENT WITH ENCEPHALITIS AND RIBAVIRIN TREATMENT FAILURE

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BACKGROUND-AIM

Neurological symptoms have also been associated with chronic hepatitis E virus (HEV) infection, including cognitive impairment, peripheral neuropathy, and encephalitis. The underlying mechanisms are not fully understood and may involve direct viral neurotropism. Therefore, we investigated within-host HEV evolution in a chronic lymphatic leukemia (CLL) patient that was diagnosed with a chronic HEV infection for over five years and clinical signs of encephalitis. The patient failed on multiple treatments with combinations of ribavirin, sofosbufivir and pegylated interferon. The aim of this study was to gain more insight in the emergence of drug-resistant variants as well as the within host evolution of HEV in the different compartments.

METHODS

A new full-genome amplicon tiling amplification protocol for HEV genotype 3 was developed based on previously published full genome amplification protocols. In total 23 available stored clinical samples were available from feces, blood and CSF. Sequencing was performed using R9.4.1 flowcells in combination with the rapid barcoding kit 96 (SQK-RBK110.96) on a Nanopore GridIon. Data analysis was performed using geneious prime version 2023.0.1.

RESULTS

For 17/23 (74%) of samples consensus sequences were obtained with a minimal genome coverage of 95% and minimal depth of 200. All failed samples had low viral loads below 10.000 IU/ml. We observed distinctive evolution in CSF compartment compared to the plasma and feces compartment. Limited evolution was observed in CSF, while in the plasma and feces compartment multiple ribavirin resistance associated mutations inlcuding K1383N, D1384G/N, Y1587F and G1634R were detected.

CONCLUSIONS

We detected distinct viral evolution in the CSF compartment compared to the plasma and feces compartment during chronic HEV infection. In the plasma compartment ribavirin resistance associated mutation emerged, while this was not seen in the CSF compartment, possibly due to the limited penetration of the blood-brain barrier by ribavirin. Future research is needed to functionally characterize the identified mutations and their role in the neurotropism of HEV.

OP 009

Emerging and re-emerging viruses

BUILDING-LEVEL WASTEWATER SURVEILLANCE LOCALIZES INTERSEASONAL INFLUENZA VARIATION

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BACKGROUND-AIM

Constantly evolving influenza virus infects ~10% of the global population each year during waves of generally unpredictable magnitude and timing. We hoped to leverage longitudinal samples acquired over consecutive years to study the feasibility of monitoring influenza in wastewater at building-level resolution between seasons.

METHODS

Influenza A was measured in dormitory sewage of a New York City college between 2021 and 2022.

RESULTS

We uncovered building-level changes in the magnitude and timing of test positivity concordant with clinical cases. We detected very little influenza A in wastewater from the fall 2021 semester. Wastewater samples collected during the fall 2022 semester, on the other hand, revealed a frequent and heterogenous occurrence of influenza. Weekly wastewater test positivity strongly correlated with New York County clinical cases (Kendall's tau = 0.56). Positive samples were not evenly distributed among buildings. One dormitory yielded more and earlier positive results than other buildings. Wastewater tests also appear coincidental with campus clinical data. Confirmed cases lived the building with the most positive wastewater results and were diagnosed on days positive wastewater was also sampled. The 2022 data stands in stark contrast to the 2021 results by revealing the greater and earlier presence of influenza. The ~10-fold increase in positive wastewater samples mirrored the ~10-fold increase in New York County confirmed clinical cases. Wastewater from 2021 and 2022 are not equally likely to be positive ($P < 0.01$). In 2021, a positive wastewater sample was found in November when local clinical cases began to rise. In 2022, a similar number of local clinical cases appeared earlier, in October, and wastewater monitoring also detected a positive sample at that time.

CONCLUSIONS

Wastewater surveillance is capable of making quantitative measurements of the magnitude and timing of seasonal influenza between years. The ability to detect such changes could be leveraged as part of the public health response. Our work represents another in a growing body of literature highlighting the potential of building-level wastewater-based epidemiology of viruses.

OP 010

Emerging and re-emerging viruses

ENABLING GENOMIC SURVEILLANCE AND OUTBREAK ANALYSIS OF MPOX VIRUS BY DEVELOPMENT OF A NOVEL AMPLICON BASED NANOPORE SEQUENCING APPROACH

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BACKGROUND-AIM

Mpox is the clinical syndrome caused by infection with the human monkeypox virus (hMPXV). After the initial description of accumulating mpox cases internationally, the first mpox case in The Netherlands was identified on the 20th of May 2022 in Amsterdam. While rapid and accurate diagnostics were immediately implemented to aid outbreak control, a relatively low-cost and fast whole genome sequencing approach was not available. Therefore, we developed a novel 2,5 kB amplicon-based Nanopore sequencing protocol and investigated its usability to characterize the initial introduction and subsequent spread of hMPXV within the Amsterdam region.

METHODS

Our sequencing approach was developed with two PCR platforms in mind: 1) the Nextgen PCR platform, which allows for ultrafast PCR amplification using custom reagents allowing one-day NGS analysis and 2) the widely used Q5 polymerase-based approach which is globally applied for genomic surveillance of SARS-CoV-2. We made our detailed protocol publicly available on https://www.protocols.io. To verify the usability of the approaches for genomic surveillance, we initially used dilutions of a single hMPXV positive specimen to provide information on robustness and sensitivity. Next, we analyzed samples from multiple epidemiologically confirmed transmission pairs as well as samples that were epidemiologically unlinked in place and/or time.

RESULTS

Using a straightforward Q5 polymerase amplification and Nanopore sequencing protocol we were able to successfully obtain full hMPXV genome sequences from hMPXV-positive specimens. Within the generated hMPXV outbreak sequences we observed evidence of ongoing viral evolution. Sequences from known transmission pairs were highly similar and were in agreement with the transmission route. We observed several previously unknown local clusters as well as identified cases related to international travel.

CONCLUSIONS

Genomic surveillance of hMPVX using our fast and relatively cheap sequencing approach generated data on hMPXV transmission which helped to guide outbreak management and public health measures.

OP 011

Emerging and re-emerging viruses

EXPLORING THE NEUROPATHOGENICITY OF EMERGING STRAINS OF WEST NILE VIRUS LINEAGE 1 AND 2

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BACKGROUND-AIM

West Nile virus (WNV) is a neurotropic mosquito-borne flavivirus, endemic in several European countries, including Italy. In 2022, the largest outbreak of WNV neuroinvasive disease (WNND) ever recorded in Italy occurred in the Veneto Region, where a newly introduced WNV lineage 1 (WNV-1) strain co-circulated with the endemic WNV-2. Aim of this study was to compare the neuropathogenicity of the two WNV lineages.

METHODS

We analyzed clinical, and virological data of human WNV cases occurring in the Veneto Region in 2022 and confirmed by the Regional Reference Laboratory (RRL). Amplicon sequencing of the WNV genome was done directly from biological specimens. WNV-1 and WNV-2 strains isolated in 2022, as well as WNV-1 Ita09, WNV-2 AUT/08, and Usutu virus Europe 1 (USUV-E1) were used in infection experiments of cortical neurons and brain organoids. WNV infection and replication was evaluated by IF, qPCR and infectious virus titration. Viral modulation of gene expression was analyzed by RNA sequencing.

RESULTS

438 human cases of WNV infection were confirmed by the RRL: 186 WNND, 210 WNV fever, and 42 WNV NAT-positive blood donors. Lineage was determined in 181 cases with WNV-1 and 82 with WNV-2. WNV-1 infection was significantly associated with increased risk of WNND ($p<0.05$, Chi square test), showing an unusual incidence as acute flaccid paralysis or Guillain-Barré syndrome. However, WNV RNA load was higher in WNV-2 than in WNV-1 WNND patients. Phylogenetic tree analysis showed that WNV-1 genomes formed a monophyletic cluster with minimal diversity. Both WNV-1 and WNV-2 strains efficiently infected and replicated in human cortical neurons and brain organoids in vitro, with higher replication efficiency of WNV-2, and up-regulated expression of genes involved in ER stress response, innate antiviral response, apoptosis induction, neurodegeneration. USUV-E1, used as control, infected and replicated in neurons with low efficiency, in agreement with the low pathogenicity observed in humans.

CONCLUSIONS

A new WNV-1 strain associated with increased risk of WNND emerged in the Veneto Region in 2022. Monitoring WNV genetic evolution and phenotypic characterization of new variants is crucial to early detect the emergence of new strains with epidemic potential.

OP 012

Emerging and re-emerging viruses

IN VITRO MODELS TO ASSESS AUTO-ABS NEUTRALIZING THE PROTECTIVE EFFECT OF TYPE I IFNS AGAINST WNV INFECTION

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BACKGROUND-AIM

Mosquito-borne West Nile virus (WNV) infection is asymptomatic or causes a mild febrile illness in the great majority of individuals, but can cause WNV neuroinvasive disease in ~1:150 infected individuals. Auto-antibodies (auto-Abs) neutralizing type I IFNs underlie ~15% of hypoxemic COVID-19 pneumonia, ~5% of critical influenza pneumonia, ~25% of MERS pneumonia and ~30% of adverse reactions to the Yellow Fever D17 vaccine strain. We recently identified auto-Abs in ~40% of individuals with WNV neuroinvasive disease. In vitro models to assess auto-Abs neutralizing the protective effect of type I IFNs against WNV infection are crucial to define their pathogenic role and screen patients.

METHODS

Circulating IgG-auto-Abs against type I-IFNs had been previously detected by ELISA in 102 sera from WNV infected subjects enrolled in Pavia. In vitro WNV infection in human retinal pigment epithelial cells (ARPE-19) was assessed using different IFN-ς2 concentrations with serial dilutions of sera from patients with WNV disease carrying auto-Abs against IFN-ς2 (n=5) and IFN-ϒ (n=4). Sera from patients with WNV disease without anti-type I IFN auto-Abs were used as controls. The experiments were carried-out in parallel on Vero E6 cells.

RESULTS

We show that IFN-ς2 and IFN-ϒ protect ARPE-19 cells from WNV infection in vitro. ARPE-19 cells show similar sensitivity to IFN-ς2 compared to Vero E6 cells. Serum samples from patients with auto-Abs block the antiviral function of IFN-ς2 and/or and IFN-ϒ in ARPE-19. In experiments to test serial dilutions, the sera maintained their blocking effect up to a 1:300 dilution, while no effect was observed using sera without auto-Abs.

CONCLUSIONS

Our preliminary data show that the human ARPE-19 cell line is as reliable cell line to test sera from patients with WNV disease for neutralizing anti-type I IFN auto-Abs. Our findings also confirm that neutralization of type I IFN antiviral immunity is a major mechanism of life-threatening viral disease. The ARPE-19/WNV cell model should be validated for testing of samples from patients with infection other than WNV, including COVID-19, influenza pneumonia and MERS. Our experiments are also a starting point to test other human cells for sensitivity to type I IFNs and the blocking effect of sera from patients with life-threatening viral disease.

OP 013

Enteroviruses

A PILOT STUDY FOR AN INTERNATIONAL SURVEILLANCE MODEL CAPTURING SEVERE NEUROLOGICAL ENTEROVIRUS AND PARECHOVIRUS INFECTIONS

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BACKGROUND-AIM

Enteroviruses and parechoviruses (EVs/HPeVs) are the causal agent for the majority of viral central nervous system (CNS-) infections. However, the burden of neurological disease caused by these viruses is unknown throughout Europe. We aim to establish a valid and reliable standardized surveillance system for EV/HPeV CNS-infections including meningitis, encephalitis, meningoencephalitis, sepsis-like syndrome, acute flaccid paralysis/myelitis and associated EV/HPeV type.

METHODS

Study sites from all over Europe will be invited and included on voluntary basis. Adult and pediatric patients with CNS-involvement and suspected viral etiology according to our case definition and inclusion criteria will be included.
Standardized questionnaires will be used to record demographic and clinical information at first medical examination, and clinical outcomes no later than 3 months after the first medical examination. For laboratory diagnostics/investigation, CSF (cerebrospinal fluid), feacal and respiratory specimens will be collected and analyzed to detect presence of EVs and HPeVs and for genotyping.
All participating sites will enter study data into a REDCap database along with the weekly number of symptomatic patients with no EV/HPeV positive tests. The database is developed and managed by the European Non-Polio Enterovirus Network (ENPEN) and hosted in Denmark.

RESULTS

Project newsletters will inform the participating sites and the rest of ENPEN about CNS-infections caused by EVs/HPeVs and about the developments in the pilot surveillance network. The first results will be presented at ESCV 2023. The first children were enrolled in Denmark in April 2023, and more cases will continue to be enrolled at hospitals in Spain, The Netherlands, Ireland, and the United Kingdom with ongoing inclusion of more participating partners. After one year of the pilot surveillance, the complete dataset will be analyzed, compared and presented and the pilot surveillance system will be evaluated.

CONCLUSIONS

Our pilot surveillance of neurological infections associated with EVs and HPeVs will gather real-time evidence on virus circulation, on their disease manifestation and severity, used to estimate the burden of these infections. The pilot surveillance system will be evaluated and considered for broader implementation across Europe.

OP 014

Enteroviruses

CLUSTER OF NEONATAL ENTEROVIRUS MYOCARDITIS CASES IN CARDIFF, UNITED KINGDOM.

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BACKGROUND-AIM

Enteroviruses can cause a range of serious clinical conditions with certain rare complications being associated with particular serotypes. In the autumn of 2022 there were 4 neonates critically unwell with myocarditis and enterovirus infection in the Childrens Hospital for Wales, United Kingdom. From the same Childrens hospital in Wales in the preceding 7 years there were only 4 cases. A cluster of 10 cases was reported to the World Health Organisation

METHODS

A retrospective review of all cases of either neonatal myocarditis or enterovirus positivity from July 2022 was undertaken. 10 cases of neonatal enterovirus myocarditis were identified and clinical and laboratory data reviewed to characterise this cluster. Of these 10 babies there was one death prior to retrieval to critical care, 2 babies were cared for on a general ward and 7 babies needed critical care support two of which were cared for outside of Wales. The babies in critical care received supportive care as well as ivIg, pocapavir and immunomodulation with steroids and anakinra (il-1 inhibitor).

RESULTS

All babies that were cared for in intensive care units survived to discharge from ICU, many had prolonged hospital admissions and as of May '23 one child remains an inpatient, 2 babies were considered for cardiac transplant or palliation.
For the 7 babies who were on critical care 5 had EDTA blood sent all of which were PCR positive, 6 had stool sent and only 4 of these were positive. EDTA blood was positive for 40 days in 1 patient.
There was significant cardiac compromise with elevated biomarkers, abnormal ECG and echocardiography.
Routine enterovirus surveillance in Wales demonstrated that enterovirus was not circulating at higher than expected levels.

CONCLUSIONS

An unusual increase in cases of neonatal enterovirus myocarditis was reported and investigated, early indicators of note are that all cases were due to coxsackie viruses and viraemia was often detected and was prolonged.

OP 015

Enteroviruses

EPIDEMIOLOGICAL AND MOLECULAR CHARACTERIZATION OF HUMAN ENTEROVIRUS IN OUTPATIENTS AND HOSPITALIZED PATIENTS WITH ACUTE RESPIRATORY ILLNESS IN LOMBARDY (NORTHERN ITALY), FROM JULY 2021 TO APRIL 2023

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BACKGROUND-AIM

Enteroviruses (EVs) cause mild-to-severe acute respiratory tract infection, with the highest morbidity in children. Some EV genotypes may cause severe and life-threatening disease. Since the lack of non-polio EV surveillance in Italy, this study aimed at investigating the circulation and molecular characteristics of EVs in individuals with mild and severe acute respiratory illness

METHODS

We considered respiratory samples collected from July 2021 to April 2023 from outpatients with influenza-like illness (ILI) in the framework of influenza surveillance activities and inpatients attending to emergency room (ER) or hospitalized with severe acute respiratory infection (SARI) in 8 hospitals in Lombardy. Samples EV-RNA-positive were tested by a real time RT-PCR assay for detection of EV-D68; molecular characterization was carried out by sequencing of a fragment of the VP1/VP3 gene of EVs

RESULTS

We analysed 497 laboratory-confirmed EV-positive samples: 55.3% from inpatients and 44.7% from ILIs. EV-positive individuals had a median age of 2 years (IQR: 3 years), 55.3% were males. 54.4% of EVs was identified from October to December and three consecutive epidemics were observed, which peaked at the end of November 2021, start of July 2022 and end of November 2022. EVD68 genome was identified in 28.2% of EV-positive samples: 72.1% of EVD68 cases were SARIs. EVD68-positive individuals had a median age of 3 years (IQR: 4.5 years), 42.1% were males. The first EVD68 wave (28.9% of EV-D68s) peaked at end of November 2021, followed by a second wave (75% of EV-D68s), peaked in October 2022. 81/353 (23%) EV-positive samples (other than EV-D68) were sequenced. Their molecular characterization revealed the co-circulation of EVs group A (65.4%: CVA2, CVA4, CVA5, CVA6, CVA9, CV16, EVA71) and group B (34.6%: E3, E11, E25, CVB5, CVB2)

CONCLUSIONS

EV-positive samples were mostly collected from young children with severe and mild respiratory infections during fall months. The co-circulation of several EV genotypes in consecutive outbreaks calls for a deepen molecular characterization by innovative technology as NGS. The set-up of a standardized surveillance system based on networking between clinical and public health virologists to monitor the spread and clinical features of EVs is a milestone to be achieved soon

OP 016

Enteroviruses

NEONATAL ENTEROVIRAL SEPSIS - CASE OF ECHOVIRUS 11 (EV-11)

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BACKGROUND-AIM

Enterovirus (EV) infections are common in neonates, but can often be associated with serious manifestations and high mortality. Severe life threatening complications including hepatic necrosis with coagulopathy, meningoencephalitis and myocarditis usually present within the first week of life. Here we present a case of a twin pregnancy (DCDA) with delivery at 34+6 of female infants who simultaneously became unwell day four of life, with rapid deterioration necessitating transfer to PICU and paediatric antiviral (Pocapavir®, ViroDefence® USA) option assessment. Mother had recent flu-like illness and a child at home under two with hand, foot and mouth disease (HFMD).

METHODS

From onset of clinical decline (day 4) infant PCR screening determined systemic enteroviral infection (skin, mucosa, respiratory, EDTA blood). Early administration of IVIG Octagam 10%, and application made for Pocapavir®. Swab samples (Cobas PCR Media tubes) and blood (EDTA) were processed and PCR amplified using an in-house Enterovirus/Parechovirus qualitative Taqman® PCR (Roche Flow®). Positive samples were reflex tested via UKHSA VP1 sequencing. No faeces of CSF were obtained.

RESULTS

Systemic enterovirus in both infants with positive pan-Enterovirus PCR results from throat swab, mucosa, and EDTA blood. PCR in EDTA blood were CT17 and CT18 in each infant respectively in comparison to CT>30 in respiratory and mucosal samples. Enterovirus typing by VP1 genotyping resulted as Human Enterovirus Species B, Echovirus E11 (E-11). Skin swabs were PCR negative.

CONCLUSIONS

Both infants deceased at day 10 of life; with disseminated enteroviral infection, multi-organ failure, disseminated intravascular coagulation, with extensive necrosis of the liver, hypocoagulopathy, anaemia and marked prolongation of the prothrombin time (120). Typing has resulted as E-11, Human Enterovirus B. NextGenSeq (NGS) and phylogenetic analysis via UKHSA awaited, as part of UK wide enhanced interest post COVID neonatal enterovirus infection. E-11 has frequently been associated with severe sepsis-like illness with hepatitis or HNC with a high fatality. The access to Pocapavir® was made as 'Emergency use in treatment of patients with Serious Enterovirus Infection' but unfortunately arrived 24h post death.

OP 017

Enteroviruses

THE ENVIRONMENTAL SURVEILLANCE FOR ENTEROVIRUSES: FROM A HISTORICAL PERSPECTIVE TO THE STATE OF ART

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BACKGROUND-AIM

Although enteroviruses (EV) are increasingly recognised as cause of severe infections in young children, most infections remain asymptomatic. Environmental surveillance is a powerful tool to monitor EV circulation, including those EV types/strains not associated with symptomatic disease, and to identify (re)-emerging virus types/strains. However, it is important to consider the purpose and methodology of environmental surveillance.

METHODS

European Non-Polio Enterovirus Network (ENPEN) organised an expert webinar to discuss history, methods and applications of environmental surveillance. Here we describe 1) the historical implementation of environmental surveillance; 2) its evolution from culture-based to molecular detection and 3) future implementation of next generation sequencing (NGS).

RESULTS

Environmental surveillance, first established in early 1960's, can identify circulation of polioviruses weeks before the first cases of polio become apparent. It was calculated that 400ml sewage sample is sufficient of detection of the virus if 1:10,000 people excrete poliovirus in a population of 700,000 people. It has also become apparent that most sewage samples contain tens of enterovirus types, if collected and tested appropriately. Although culture-based methods are still the golden standard for poliovirus-surveillance, direct methods followed by Sanger, Illumina or Nanopore sequencing have been recently developed.

CONCLUSIONS

Understanding the extend and quality of environmental surveillance utilised to study enterovirus circulation across the European region is important. ENPEN coordination of shared protocols and database will enforce the good quality of environmental surveillance leading to meaningful data in the region. It will also help to detect the (re)-emerging enterovirus types/strains.

OP 018

Gastrointestinal viruses

ANALYTICAL AND CLINICAL VALIDATION OF A LAB-DEVELOPED, FULLY AUTOMATED HIGH-THROUGHPUT MULTIPLEX-PCR PANEL FOR THE DETECTION OF SEVEN GASTROINTESTINAL VIRUSES: NOROVIRUS GT1, NOROVIRUS GT2, ROTAVIRUS, ADENOVIRUS, SAPOVIRUS, ASTROVIRUS AND ENTEROVIRUS

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BACKGROUND-AIM

Diarrheal illness is a driver for emergency department visits and hospitalization in Europe and worldwide; frequently caused by a range of gastrointestinal viruses. Rapid diagnostics can limit duration of isolation measures and may help limit length of hospital stay and guide treatment. The SARS-CoV-2 pandemic served as a catalyst for widespread adoption of fully automated high-throughput PCR platforms.
The aim of this study was to develop, validate and implement a multiplex-RT-PCR panel for detection of 7 gastrointestinal viruses on the open channel of one such platform (cobas5800/6800/8800).

METHODS

RT-PCR assays were selected from literature and modified for inclusivity and compatibility, targeting Norovirus (NoV) GT1&2, Rotavirus (RV), Adenovirus (AdV), Sapovirus (SaV), Astrovirus (AsV) and Enteroviruses (ENV).
Stool was swabbed and transferred into 40% guanidine hydrochloride solution. Analytic performance was evaluated using quantitative reference material generated by digital PCR. Analytic LoD (lower limit of detection), linearity and precision were determined by serial dilution of reference material or clinical samples. Up to 243 predetermined samples were assessed for clinical performance.

RESULTS

The novel multiplex panel demonstrated good analytic sensitivity (LoD, 95% probability of detection) for: NoV GT1: 3180 cp/ml, NoV GT2: 299 cp/ml, RV: 851 cp/ml, AdV: 54.6 cp/ml, SaV (GT1-4): 57 cp/ml, AsV: 65.4 cp, and pan-ENV: 29.4 cp/ml; and good linearity (verified by serial 10-fold dilution over 4-8 log steps, with pooled SDs (Ct) over linear range: NoV GT1: 0.33, NoV GT2: 0.26, RV: 0.2, ADV: 0.18, SapV: 0.06, ASV: 0.35 and ENV: 0.27. Precision was verified over 3 consecutive days. Empirical exclusivity testing yielded no false positives in a set of 51 samples containing different viral and bacterial species.
Clinical performance testing revealed positive agreement for NoV GT1 of 100% (n=243, 3 pos), GT2: 95.7% (n=241, 47 pos), RV: 100% (n=207, 18 pos) and AdV: 96.6% (n=177, 29 pos). Assessment for SaV, AsV and ENV is ongoing.

CONCLUSIONS

The assay panel we present here allows for easy adaptation of automated testing infrastructure for a range of common gastrointestinal viruses and shows excellent analytic performance.

OP 019

Gastrointestinal viruses

DETECTION OF VIRAL AGENTS CAUSING GASTROENTERITIS BY MULTIPLEX PCR METHOD IN PATIENTS; NOROVIRUS, ROTAVIRUS, ADENOVIRUS, ASTROVIRUS, SAPOVIRUS

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BACKGROUND-AIM

Acute viral gastroenteritis may lead hospitalization and death in individuals with underlying health conditions such as immunosuppression. Identification of pathogen causing gastroenteritis is important for appropriate treatment. It is important to demonstrate the presence of the viral nucleic acid by real-time polymerase chain reaction (RT-PCR) in the stool specimens of the patients for the laboratory diagnosis of viral gastroenteritis. The aim of this study was to determine the frequency of viral gastroenteritis and evaluate the distribution and characteristics of viral agents among patients hospitalized with gastroenteritis at a university hospital from June 2019 to December 2022.

METHODS

A total of 340 stool samples from 265 patients with gastroenteritis submitted to the Virology Laboratory were included in the study. The gastrointestinal syndromic (GIS) panel was performed on stool samples of the patients.

RESULTS

Of the 340 samples, 121 (35%) were positive. Viral agents were detected in 21 (%17) of these positive samples. One hundred and seventy three samples were sent from immunocompromised patients with transplants, human immunodeficiency virus (HIV) infection, and malignancies etc. Among these, 47 (27%) samples were positive by GIS panel. When comparing the norovirus frequency versus the other viral agents, norovirus was the most viral agent detected in samples from immunocompromised patients. Rotavirus A was detected in two stool specimens, but other viral agents were not detected in any of the stool specimens of the immunocompromized patients. Immunosuppression was found as a risk factor for norovirus gastroenteritis in this study.

CONCLUSIONS

Early diagnosis of viral gastroenteritis in immunocompromised patients is crucial to reduce transmission. Therefore, the use of a GIS panel in these patients for the diagnosis of viral gastroenteritis should become common.

OP 020

Gastrointestinal viruses

GENETIC DIVERSITY OF GROUP A ROTAVIRUS CIRCULATING IN IRELAND BETWEEN 2015-2021 IDENTIFIED THROUGH NANOPORE WHOLE-GENOME SEQUENCING

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BACKGROUND-AIM

Group A rotavirus (RVA) commonly causes paediatric gastroenteritis. Despite vaccine availability in 123 countries, there remain ~200,000 childhood deaths per year from rotavirus disease, primarily in low-income countries. Genotypic surveillance is important to monitor viral diversity and identify potential vaccine-escape strains. The segmented dsRNA RVA genomes are usually found in one of three genotype constellations referred to as: Wa-like, DS-1-like and Au1-like. Reassortant viruses with mixed constellations are also reported.

METHODS

Between 2015-21, RVA was detected in 2100 samples from patients tested at the NVRL. G- and P-types were determined by RT-PCR (n=1120) and 157 samples were processed for whole-genome sequencing (WGS). Following viral enrichment and amplification (NetoVIR), libraries was prepared for nanopore sequencing. Genotypes were assigned using www.bv-brc.org.

RESULTS

G- and P-typing identified that G1P[8] was most prevalent in 476/1120 (42%) of samples, followed by G9P[8] (16%), G2P[4] (14%), G4P[8] (10%), G3P[8] (7%), G12P[8] (2%) and infrequent/mixed/untypable genotypes (7%). Of the samples processed for WGS, 139/157 (89%) generated data for all 11 segments. Notably, ten (7%) were reassortant viruses with eight distinct genome combinations. All ten were from patients tested in 2018-19, aged 1-72 years (median 6 years). In particular, 14 (10%) were either G1P[8], G3P[8] or G8P[8] with the atypical DS-1 genotype constellation. These samples were from 2016-19, patients aged 8 months-85 years (median 2 years).
In addition, 94 samples (68%) identified as G1P[8], G3P[8], G4P[8], G9P[8], G12P[6] or G12P[8] were found with the typical Wa-like genotype constellation, whereas 15% were either G2P[4], G3P[4] or G9P[4] associated with the DS-1 genotype constellation.

CONCLUSIONS

RVA G- and P-typing showed similar distribution of genotypes circulating in Ireland to those reported elsewhere in Europe. WGS analysis revealed 17% viruses were reassortant or demonstrated an atypical genotype constellation. Genetic reassortment may be underreported due to fewer laboratories performing RVA WGS which the cost-effective method presented here may help address. The infectious and epidemiological consequences of such reassortment merit further investigation.

OP 021

Gastrointestinal viruses

PREVALENCE OF GASTROINTESTINAL PATHOGENS BY MULTIPLEX PCR GASTROINTESTINAL PANEL STOOL TEST IN PATIENTS WITH AND WITHOUT INFLAMMATORY BOWEL DISEASE

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BACKGROUND-AIM

The aim of this study was to investigate the distribution of gastroenteritis agents in diarrhea patients with and without inflammatory bowel disase(IBD) with the GI panel test and also to evaluate its potential effect on infection control and patient prognosis by providing early diagnosis of the causative agent of gastroenteritis.

METHODS

Our study is based on a retrospective cohort analysis. Between June 2019 and December 2022, 266 patients with diarrhea whose stool samples were studied with multiplex gastrointestinal panel(GIP) test in Gazi University,School of Medicine,Medical Virology Laboratory were included in the study.Data collected had the patients' age,sex,presence of ulcerative colitis(UC) or Crohn's disease(CD) and results of stool culture,microscopic examination and gastrointestinal pathogen PCR stool test.

RESULTS

Two hundred twenty six patients with diarrhea underwent 339 GI panel tests.Among the 266 patients studied,thirteen patients were determined as IBD.Of the 13 IBD patients,8 of them had CD whereas 5 had UC.One or more pathogens were found in 101 of 266 patients by GIP testing.A total of 154 enteric pathogens were detected in 101 patients.EPEC(22.8%) was the most common pathogen.The test result was positive in 5(5/13) of the IBD patients;three were CD patients and two were UC patients.EPEC(3/5) was also detected as the most common pathogen in IBD patients.In addition,ETEC,EAEC,C. difficile and C. parvum pathogens were detected but no viral pathogen was detected in IBD patients.We detected 21(13.6%) viral agents in patients without IBD.We detected enteric virus pathogens including 12(%7.8) Norovirus, 4(%2.6) Rotavirus, 2(%1.3) Astrovirus, 2(%1.3) Sapovirus, and 1(%0.8) Human Adenovirus F40/F41,respectively.When the distribution of patients according to IBD subtypes and gender was examined, no statistically significant difference was found.

CONCLUSIONS

Fewer viral and parasitic pathogens were found in IBD patients tested with GIP tests, possibly due to non-infectious diarrhea and altered immunity.We think that GI viral pathogens are not effective in IBD.Rapid identification of any pathogen offers a chance for treatment, making PCR-based testing advantageous. In addition, a negative result of GIP test is of great significance for the clinician to guide the treatment.

OP 022

Hepatitis/HIV

CHARACTERIZATION AND ROLE OF HIV-DNA MINORITY MUTATIONS IN A LONG-TERM TREATED PAEDIATRIC PATIENTS COHORT

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BACKGROUND-AIM

The aim of the study was to better understand the impact of HIV-1 minority mutations to guide the best personalize antiretroviral treatment (ART) in paediatric patients (pts).

METHODS

An observational study was carried out during the period from August 2022 to March 2023 at IRCCS Bambino Gesù Children's Hospital among 18 paediatric/young adults pts with HIV-1 diagnosis. HIV-RNA and total HIV-DNA were evaluated and Next Generation Sequencing (NGS) was performed on HIV-1 positive samples. The presence of minority resistance mutations (mRMs) (frequency of 5-20%) and signature APOBEC-related mutations (APO-Ms) was evaluated.

RESULTS

The enrolled pts were mainly female (10,55.6%) with a median(IQR) age of 18(11-24) years. All pts were followed in our hospital since the first diagnosis, three of them received a HIV-1 diagnosis during the analysis period and thus were naïve-treated. Fourteen(77.8%) pts had an undetectable or <200 cp/mL of HIV-RNA, the remaining 4 pts, had a median(IQR) HIV-RNA of 43272(16373-488497) cp/mL. The HIV-DNA load was detectable for all pts with a median(IQR) of 1435(553-3503) cp/106 CD4+T-cells.
Nine pts(50%) received an early ART with a median duration of 10 years (IQR 6-18). The majority of pts (16,88.9%) had received Nucleoside Reverse Transcriptase inhibitors (NRTi) and Integrase inhibitor (INI) regimen, 16.7% (N=3) Protease Inhibitors (PI) regimen and 11.1% (N=2) Non-Nucleoside Reverse Transcriptase inhibitors (NNRTi) regimen.
Looking at drug resistance mutation in HIV-DNA, 10 (55.6%) pts had at least one HIV-1 major RMs, in particular the NRTI, NNRTI and PI resistance were present in 27.8%, 22.2% and 5.6% of cases, respectively. Eleven (61.1%) pts were detected to harbour at least one HIV-1 mRMs, mainly localized in RT region followed by Integrase and Protease regions. Half of patients showed at least one APO-Mutation and only one APO-related stop codon at a frequency ranging 3-56%.

CONCLUSIONS

This study demonstrated that despite the prolonged and in some cases early treatment the HIV-1 reservoir results in minority and APOBEC-related mutations. Further analysis is needed to understand whether these mutations are the evolutionary adaptation of the virus under pharmacological pressure that could affect viral fitness and guide toward personalized therapy.

OP 023

Hepatitis/HIV

DOWNTREND HCV AND HBV VIREMIA PREVALENCE IN DIALYSIS PATIENTS IN SOUTH BULGARIA

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BACKGROUND-AIM

Hemodialysis patients are at an increased risk of hepatitis C (HCV) and hepatitis B virus (HBV) infections. Prevention of their transmission remains a challenge worldwide.
We aimed to analyze the viremia prevalence of HCV (HCVv) and HBV (HBVv) in dialysis patients.

METHODS

We performed a cross-sectional study in 3 dialysis units in South Bulgaria between January 2020 and July 2022 and enrolled 225 patients. Serological markers for HBV and HCV were detected with ELISA. HCV-RNA and HBV-DNA were determined quantitatively by commercial PCR.

RESULTS

The median age of the patients was 64 years, and 56% were male. Of the 225 cases, 14 tested anti-HCV-positive, and another 12 were diagnosed with chronic HBV infection. Of the latter, six were eligible for antiviral treatment. Five of them commenced therapy, and one later died. Another patient who refused treatment also died. Consequently, four cases commenced treatment and remained virologically suppressed. The remaining six patients with chronic HBV infection had low-level viremia (<2000 IU/mL) and did not require treatment. Two of them survived, resulting in an HBVv prevalence of 0.9%.
Among the 14 anti-HCV-positive cases, five had received direct-acting antivirals (DAAs) a few years ago and maintained sustained virologic response (SVR). Of the remaining nine patients, 3 were repeatedly tested negative for PCR RNA, while the other six were HCV RNA-positive. Three of these six cases received DAAs and achieved SVR. One patient died before the initiation of DAAs. Two other viremic patients with severe comorbidities declined to move to another hospital setting until DAAs were administered. They accounted for an HCVv prevalence of 0.9%. The remaining 221 anti-HCV-positive cases tested HCV-RNA negative.

CONCLUSIONS

Our results show that the viremia prevalence of both HCV and HBV is very low. However, HBV treatment is almost lifelong. Even when no treatment is needed, long-term monitoring is necessary. Conversely, the low HCVv prevalence is the result of short curative DAAs treatment. Eradication of HCV seems feasible by screening strategies, promoting easy access to DAAs, and strict infection control practices.

OP 024

Hepatitis/HIV

MOLECULAR CHARACTERISATION OF A RABBIT HEPATITIS E VIRUS STRAIN DETECTED IN A CHRONICALLY HEV-INFECTED INDIVIDUAL FROM GERMANY

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BACKGROUND-AIM

Immunocompromised individuals, such as transplant patients, are susceptible to chronic hepatitis E virus (HEV) infections due to persistent viremia. HEV can be transmitted from pigs and wild boars to humans through zoonotic means. Recent reports have also confirmed sporadic infections with rabbit HEV (raHEV) strains in humans. In this study, a raHEV strain isolated from a heart-transplanted patient who was chronically infected with HEV was characterized at the molecular level using a novel whole viral genome sequencing approach.

METHODS

The patient had previously undergone successful ribavirin (RBV) treatment for chronic HEV infection in 2019, but tested positive for HEV again in 2021. As a result, the patient received a second cycle of RBV therapy. Viral RNA extracted from a plasma sample taken between treatment cycles, as well as a stool sample taken two months into the second cycle were subjected to full-length HEV genome amplification using two overlapping PCRs and subsequent next-generation sequencing. Phylogenetic and mutational analyses were performed using the sequencing data.

RESULTS

Deep sequencing of the plasma (raHEV-83) and stool (raHEV-99) samples and subsequent phylogenetic analysis revealed the highest nucleotide sequence identity with a Chinese raHEV strain. Additionally, a phylogenetic relationship was observed between these strains and a French patient raHEV strain. Mutation analysis also revealed the presence of RBV-associated substitutions in the RdRp, including V1479I and G1634K in plasma and stool, and additionally K1398R in the stool sample.

CONCLUSIONS

Our study detected a raHEV strain in a heart-transplanted patient from Germany and underline the role of raHEV in human infection. Although human infections with raHEV are rare, they emphasize the importance of a one health approach to comprehensively understand the epidemiology of HEV in both humans and animals, and to facilitate prevention and control of HEV infections. The study highlights the importance of sensitive methods to detect patient-specific viral variants that can impact treatment outcomes, and has implications for future one health, diagnostic, prognostic, and personalized medicine concepts.

OP 025

Hepatitis/HIV

ULTRA-SENSITIVE HBV DNA DETECTION AND RISK PREDICTION OF OCCULT HEPATITIS B VIRUS INFECTION IN BLOOD DONORS IN ENGLAND

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BACKGROUND-AIM

Occult hepatitis B virus infection (OBI) accounts for much of the residual risk of HBV transmission and is characterised by low viral loads. We aimed to develop an ultra-sensitive PCR system for HBV DNA detection and investigate risk factors associated with DNA presence in English anti-HBc blood donations.

METHODS

Seven extraction methods of differing extraction volumes combined with three qPCR assays were compared across 26 HBV-positive plasma samples. The optimal system was utilised to determine DNA presence in anti-HBc positive and anti-HBs < 100 IU/L donations identified from the introduction of anti-HBc screening. Including further data from OBI donors identified since HBV DNA screening in 2009, serological data were compared between DNA-positive and DNA-negative donors.

RESULTS

Extraction from 5mL of plasma increased sample representation and resulted in the detection of HBV DNA in low viral load samples (~0.5 IU/mL). Screening of 487,763 donations between May and December 2022 identified 2042 anti-HBc positive donors and 412 with anti-HBs < 100 IU/L. Testing 134 of the latter donations utilising the 5mL extraction method identified two further DNA positives in addition to two OBI donors identified from routine screening. Higher anti-HBc titre and anti-HBs negativity were significant predictors of DNA detectability in anti-HBc donations.

CONCLUSIONS

We developed an ultrasensitive PCR assay for HBV DNA detection which was able to identify further OBI donations increasing our detection rate from 0.5% (2/412) to 2.9% (12/412). The utilisation of anti-HBc titres may further complement the risk stratification of DNA-positive donors in anti-HBc screening and minimise deferred donations.

OP 026

Immune response and vaccines

CONVALESCENT PLASMA TREATMENT OF COVID-19: ANTIBODY PROFILES OF DONORS AND PATIENTS

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BACKGROUND-AIM

Convalescent plasma (CP) treatment of COVID-19 has shown significant therapeutic effect when administered early but failed to show clinical benefit when treating those more severely ill. We have investigated whether properties in the CP used could explain these differences, and further measured the effect of CP in the antibody levels of recipients.

METHODS

Antibody properties were measured in plasma used in the unsuccessful REMAP-CAP (n=56) and successful Argentinian trials (n=46), collected from convalescent vaccinees (n=102), and in the REMAP-CAP patients (n=82). In-house ELISA was used to measure IgG titres and avidity, and microneutralisation for nAb titres.

RESULTS

Although the mean IgG titre was 1.7-fold higher in the REMAP-CAP than in the Argentinian plasma (1952 versus 1147, $p<0.05$), no difference in nAb titres was observed. Vaccinee plasma showed highest IgG and nAb titres (11,539 and 747), and over 2-fold greater avidity than plasma used in trials (0.58 versus 0.19 and 0.25, respectively; $p<0.05$). Median nAb titres increased to 1090 in seronegative CP recipients (n=20) whereas no increase in nAb titres was seen in those with nAb prior to CP transfusion (n=62; 1171 to 2260).

CONCLUSIONS

We found no difference between the trial plasmas but noted a 1000-fold increase in nAb levels in seronegative patients compared to seropositives, emphasising initial patient serostatus as treatment efficacy predictor. Low IgG-avidity with IgM-positivity in the CP used in both trials was typical for endogenous antibodies produced during primary infection; vaccinee plasma with higher titres and avidity should be preferable in future.

OP 027

Immune response and vaccines

LOW IMMUNOGENIC RESPONSE TO SARS-COV-2 VACCINE-BOOST IN CANCER PATIENTS: CASE SERIES FROM PORTUGAL

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BACKGROUND-AIM

Cancer patients (including haematological malignancies) are at higher risk of complications and death following infection with acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Vaccination programmes were expected to increased protection of these patients and therefore booster doses are proposed to increase response to SARS-CoV-2. We aimed to evaluate the immunological response of cancer patients to booster doses of the vaccine and stratify the patients accordingly.

METHODS

The study included cancer patients (solid and haematological tumours) attended at the Portuguese Oncology Institute of Porto (IPO Porto) that received SARS-CoV-2 vaccine-boosts: 1) cohort A with 54 patients collected samples before the booster dose and 3- and 6-months after (44 solid and 10 haematological tumours); cohort B with 71 patients collected samples 3- and 6-months after booster dose (45 solid and 26 haematological tumours). A group of healthy individuals (n=83) that received SARS-CoV-2 vaccine-boosts was also analysed at 3- and 6-months post-booster dose. Humoral immune response to SARS-CoV-2 vaccine-booster dose was evaluated in serum samples analysing IgG levels against SARS-CoV-2 Spike(S) protein. To address previous contact with SARS-CoV-2 we also analysed the IgG levels against SARS-CoV-2 Nucleocapsid(N) protein.

RESULTS

Cohort A patients with solid tumours had IgG S levels increased 3 months after boost ($p<0.001$) which remained high at 6 months post-boost ($p<0.001$) when compared to pre-boost. Contrarily, patients with hematologic tumours demonstrated a weak response to vaccination since IgG S levels remained very low 3 and 6 months after the boost. When comparing 3 months post-boost in cohort A,B and healthy individuals, we observed that the healthy individuals had the strongest IgG S response, followed by the solid and, lastly, the hematologic tumours. In fact, the difference between solid and haematological tumours was quite significant ($p=0.017$).

CONCLUSIONS

We verified that the type of tumour may influence COVID-19 vaccination efficacy since solid cancer patients had a better response to SARS-CoV-2 vaccination when compared to haematological cancer patients. Moreover, as expected, healthy individuals have a stronger immune response when compared to cancer patients.

OP 028

Immune response and vaccines

THE PROTECTIVE MECHANISM AGAINST COVID-19, ANTIBODY VS CELLULAR IMMUNITY

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BACKGROUND-AIM

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of coronavirus disease 2019 (COVID-19). COVID-19 is a new major health crisis with highly distributed etiological agents globally, causing a respiratory illness with a wide range of symptoms and affecting people of all sexes and age groups, which is categorised as a pandemic by the WHO and CDC. The zoonotic pathogen was firstly discovered in Wuhan, China and has currently presented in more than 220 countries, infected more than 765 million people, with more than 6,9 million deaths due to severe respiratory illness and multi-organ failure. This study aims to clarifies the immunological features of SARS-CoV-2 for better understanding the mechanisms and functions of the immune system during infection for the purpose of the prevention and control of COVID-19 pandemic as well as for designing effective and reliable therapeutic vaccines lasting for long-term.

METHODS

To clarify the role of B and T cells as well as the inflammatory cytokines production in COVID-19, we systematically searched Embase, PubMed, Cochrane library and Web of Science to identify and select related studies with the keywords “COVID-19”, “B cells”, “T cells” and “cytokines”. The data were measured as the mean with 95% confidence interval (CI) by Review Manager 5.3 software. The risk of bias was assessed for each selected study utilising appropriate checklists.

RESULTS

Defensive immune responses to SARS-CoV-2 are typically the result of a combination of B and T lymphocyte effector cells with protective immunity lasting between 6 and 12 months. Despite high levels of CD4+ and CD8+ T cells, Total lymphocytes, and NKCs among recovered individuals, a reduction in these cells following infection has been detected in severe disease. An increase in the secretion of cytokines markers (e.g. IFN-I, II and III, IFN-©, TNF-◊, VEGF, HGF, MCP-1, IP-10, GM-CSF, G-CSF, IL-1, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13 and IL-17) are also observed and investigated.

CONCLUSIONS

Despite challenges in developing effective, durable and stable vaccines, a deeper understanding of the vital role of B and T cells in addition to cytokines expression in COVID-19 may help in managing, controlling, and halting this new pandemic.

OP 029

Immune response and vaccines

TIXAGEVIMAB + CILGAVIMAB AS PRE-EXPOSURE PROPHYLAXIS FOR SARS-COV2 IN IMMUNOCOMPROMISED PATIENTS.

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BACKGROUND-AIM

Infection by SARS-CoV2 has become a challenge,especially for immunocompromised patients.A weaker humoral response to COVID-19 vaccine has been found in these individuals.Tixagevimab+cilgavimab(Evusheld)is a combination of human monoclonal antibodies that can be used for pre-exposure prophylaxis to prevent infection or disease by SARS-CoV2.The aim of our study was to investigate the persistence of serum antibodies and effectiveness of Evusheld.

METHODS

Immunocompromised patients were enrolled in March-September 2022.All patients had anti-spike IgG antibody levels below the threshold of 250 BAU/ml before administration of Evusheld.Patients were monthly monitored with nasal swabs and questionnaires about COVID-19 symptoms and underwent blood tests for anti-S and anti-N antibody titers every 2 months up to 8 months follow-up.

RESULTS

We enrolled 43 patients:8 lung and 5 kidney transplant recipients,12 HSCT recipients and 18 patients with autoimmune diseases.The median age was 59 years(18-82).SARS-Cov2 infection was diagnosed in 14/43 patients(32.5%) after a median time of 130 days(19-235) since administration of Evusheld. 13/14 infected patients(93%) developed symptoms, mainly fever(70%),rhinorrhea(70%),sore throat(61%) and cough(54%).Hospitalization was required in 2/14 patients(14%),and only one death occurred,although not caused by SARS-CoV2 infection.The median length of infection was 14 days(7-23).Anti-S IgG antibodies showed an increase in all patients at two months,followed since month 4 by a progressive decrease,which became more evident at month 6,as confirmed by statistical analysis. 8/14 infections(57%)occurred after 5 months since prophylaxis.For Ig anti-N titer, there were no statistically significant variation during follow-up.No significant differences were seen between infected and non-infected patients for both anti-S and anti-N antibodies. In the infected population, anti-N antibodies did not shift to positive values as expected.

CONCLUSIONS

After Evusheld administration,high levels of anti-S IgG persist for 4 months.The pre-exposure prophylaxis did not prevent SARS-CoV-2 infection in immunocompromised patients,although most infections occurred after decline of anti-S IgG levels.However,the great majority of infections were mild.

OP 030

Neurovirology

A RECENT SURGE OF SUBACUTE SCLEROSING PANENCEPHALITIS (SSPE) IN ALGERIA, 2022-2023

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BACKGROUND-AIM

Subacute sclerosing panencephalitis (SSPE) is a rare but fatal late progressive neurological disorder following measles infection. Between 2017 and 2019, there was an extensive measles outbreak throughout Algeria with more than 10 000 cases reported. Five years after, an increased number of children with neurological symptoms in favour of SSPE were observed.
Here, we present laboratory data of cerebrospinal Fluid (CSF) and serum samples related to SSPE cases.

METHODS

From January 2022 to April 2023, 70 samples (35 CSF and 35 sera) were collected simultaneously from 35 patients with suspected SSPE and sent to our laboratory for investigation.
Specific IgG against Measles Virus (MeV) were titrated in the corresponding serum and CSF samples using an ELISA kit (Euroimmun, Germany) according to the manufacturer's recommendation. Total IgG and albumin (Alb) concentrations were determined as mg/L in serum and CSF samples with nephelometric method.
Results obtained from these tests were used to calculate Ratios of MeV antibodies (QMeV), total IgG (QIgG) and albumin (QAlb). Data were evaluated by reference to the upper limit line (QLim) of the CSF/serum quotient diagrams according to Reiber method. The measles-specific antibody index (AI) was calculated either with AI=QMeV /QIgG if QIgG < QLim or AI = QMeV /QLim if QIgG > QLim. A value of ε 1.50 was considered as pathological.

RESULTS

Among 35 cases analyzed, a total of 18 patients were confirmed (12 male and 6 female). Patients presented at a median age of 4 years (range 3.5-6 years). All had contracted measles during the period of the 2017-2019 outbreak in early infancy with median latency period of 3 years and 6 months (range 3 years and 2 months-5 years). All the samples had an AI value greater than 1.50 and vary between 2.9 and 55.5.

CONCLUSIONS

Our study highlights the importance of demonstrating intrathecal measles antibody synthesis as a tool for establishing diagnosis of the disease.
In contrast to other studies, in this cohort of cases, the average time to onset of SSPE after measles was shorter and at younger ages.
As key public health message, this report indicate that Algeria is facing an increase in rate of SSPE and serve as a reminder that routine vaccination remains the best approach for preventing SSPE.

OP 031

Neurovirology

EVALUATION OF CHEMOKINES IN SERUM AND CEREBROSPINAL FLUID OF CHILDREN WITH TICK BORNE ENCEPHALITIS.

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BACKGROUND-AIM

Chitinase 3-like-1 (CHI3L1), also known as YKL-40, has been recognized as a potential marker in various neuroinflammatory processes, where it acts as a mediator of inflammation and immune response. In conditions such as Alzheimer's disease, multiple sclerosis and traumatic brain injury, increased CHI3L1 expression has been observed, suggesting its involvement in the pathogenesis and progression of these disorders. However, exact mechanisms and potential therapeutic implications of CHI3L1 in brain pathologies remains uncertain. The aim of our study was to assess the influence of various pro- and anti-inflammatory cytokines that could affect the production of CHI3L1.

METHODS

Twenty two children diagnosed with tick borne encephalitis (TBE) hospitalized in the Department of Pediatric Infectious Diseases in Bialystok, Poland were included in this preliminary study. Patients were divided into 2 groups: patients with encephalitis (n=6) and meningoencephalitis (n-=16) as assessed with the numeric scale. The permeability of the blood–brain barrier denoted as index, was calculated with Q-protein/Q-albumin. Concentration of CHI3L1, CCL2, chemerin, CXCL2, IFN-gamma, IL-1 beta, IL-4, IL-6, IL-13, TNF-alpha in serum and cerebrospinal fluid (CSF) were measured with Luminex Multipex Assay according to the protocols before and after treatment (T1 vs T2).

RESULTS

Chitinase 3-like-1 index T1 was found to be decreased in the CSF of children with encephalitis compared to the meningitis cases (median, 0.8; IQR,0.4-0.9 vs. median 1.8; IQR,1.0-2.5; p=0.011). The analysis of CHI3L1 index also showed a significant decrease in T2 vs T1 in case of encephalitis (p=0,046), but it remained constant in meningitis. Strong correlation between CHI3L1 and concentration of CXCL2, IL-6, chemerin, TNF-alpha and IL-4 in CSF (accordingly p=0,021, p=0,006, p=0,035, p-0,024, p=0,007) was observed. Interestingly, IL-6 was showed a reduction of 24 times comparing to the levels observed before the treatment.

CONCLUSIONS

Our study shows that CHI3L1 is involved in modulating inflammatory and immune responses in TBE. In encephalitis the CHI3L1 index was lower than in meningitis cases, which might suggest that its immunomodulatory effect reduces the process of neuroinflammation.

OP 032

Neurovirology

VIRAL AND HUMAN TRANSCRIPTOMIC INVESTIGATION OF CEREBROSPINAL FLUID SPECIMENS FROM PATIENTS WITH HERPES SIMPLEX VIRUS AND VARICELLA-ZOSTER VIRUS INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

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BACKGROUND-AIM

Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV) are human neurotropic alphaherpesviruses that can cause meningitis and encephalitis. The previous RetroAlpha 14-18 study aimed to investigate epidemiological, clinical, biological, and therapeutic characteristics of alphaherpevirus infections of the central nervous system (CNS) among adolescent and adult patients in France from 2014 through 2018. The goal of the present ancillary study was to characterize viral gene infection profiles and host responses at the transcriptomic level among patients with HSV or VZV CNS infections.

METHODS

The RetroAlpha 14-18 study (Ethics Committee n° CER-2021-013) has included patients with previously identified HSV-1, HSV-2 or VZV CNS infections. In addition, cerebrospinal fluids (CSFs) collected at the same period and without any identified pathogen by metagenomics were included as not infected control patients. Shotgun metagenomics (SMg) on CSF samples (DNA and RNA) was performed using NovaSeq®6000 (Illumina) and sequences were analysed using MetaMIC® software.

RESULTS

A total of 340 CSF samples (97 HSV-1, 60 HSV-2, 129 VZV, and 54 negative controls) were analyzed. The principal component and heatmap analyses of the host transcriptomics, classified the infected patients in a single group with no difference between them, while the group of non-infected patients showed a distinct signature. The difference between the two groups explored by ontology revealed a modification in the expression of the interferon and antigen presentation pathways. Viral transcriptomics showed significant differences with both lower replication and transcription for HSV-2 compared to HSV-1 and VZV; but also a marked difference in the viral transcript expression profile comparing HSV-1 and HSV-2.

CONCLUSIONS

The SMg approach on CSF allows transcriptomic profiling of the virus and the host in the context of CNS infections due to alphaherpesviruses. The host response appears to be similar regardless of the virus involved, i.e. mobilization of innate immunity associated with inhibition of antigen presentation compared to uninfected patients. Conversely, HSV-1 and HSV-2 seem to behave differently suggesting that they could have a different pathophysiology.

OP 033

One Health

DETECTION OF INFLUENZA A H5N1 IN EURASIAN OTTERS SCREENED AS PART OF A PUBLIC HEALTH SAFETY MEASURE PRIOR TO RESEARCH AUTOPSY – A POSSIBLE APPROACH FOR THE PASSIVE SURVEILLANCE OF AVIAN TO MAMMAL SPILLOVER EVENTS.

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BACKGROUND-AIM

Spillover events of influenza A into wild mammals have been reported, replication may lead to adaptations that favour infection in the human host. Currently, there is no systematic surveillance of wild mammals, so the scale of spillover isn't clear.
The Otter Project uses deceased otters as an indicator for environmental health. The otter project in collaboration with the National Influenza Centre for Wales, developed a sampling protocol to screen otters for influenza A and SARS-CoV-2 prior to formal autopsy.

METHODS

Otters are submitted frozen and allowed to defrost for 24 hours to enable sampling of the nose, rectum and trachea. The swabs are immediately placed in lysis buffer and then transported to the laboratory for screening by influenza A PCR targeting matrix gene and SARS-CoV-2 PCR targeting the E gene. Samples that test positive for influenza A are subtyped using assays for seasonal influenza A H3 and H1 and avian H5 and H7.
Otters that screen negative can then proceed to autopsy, where a swab of the brain is collected for influenza A testing.
Whole genome sequencing is attempted on samples with good yield of RNA.

RESULTS

Three out of 84 otters have screened positive for influenza A, no otters have tested positive for SARS-CoV-2. Otter 1 found in 2020 was positive in trachea only. Due to the condition of the body this result could not be confirmed.
Otter 2 found in May 2022, in a region with wild bird die off, was positive in brain only, it was successfully typed to influenza A H5.
Otter 3 found in January 2022, in a region with no reported avian influenza activity, was positive for Influenza A H5 in trachea and brain. The viral load allowed for successful sequencing and H5N1 clade 2.3.4.4b confirmed. An initial screen on nasal and rectal samples by another laboratory was negative. All of the people involved in the autopsy were offered prophylaxis and monitoring.
Due to SAPO4 regulations, the otter remains were transported to the Worldwide Influenza Centre in London.

CONCLUSIONS

This work acts as a passive surveillance scheme for two viruses of public health concern, giving evidence of spillover occurring into wild mammals, even in areas with no reported avian influenza outbreaks.
The methods used could be adapted for similar schemes to support this area of one world health.

OP 034

One Health

SURVEILLANCE OF SARS-COV-2 VARIANTS IN WASTEWATER OF AIRPLANES

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BACKGROUND-AIM

Air travel plays a key role in the global spread of many diseases including COVID-19. Surveillance of international travelers for SARS-CoV2 is useful for the detection of emerging variants, especially in a context of global relaxations in testing policy.
We tested whether wastewater surveillance of aircrafts can be used as a low-cost mechanism to monitor SARS-CoV2 variants entering Belgium, especially from countries were data and information on variants is low.

METHODS

Over a period of 3 months in 2023, 32 wastewater samples were collected from direct flights coming from China. Since a decontamination fluid is used in airplane toilets, detection of the human fecal control pepper mild mottle virus (PMMoV) was used as a positive control. Different qPCR's, panels and sequencing method were performed during this pilot project.

RESULTS

PMMoV was detectable in all samples. Nineteen samples tested positive for SARS-CoV-2 (Ct 25.8 - 37.5). The use of a respiratory panel and a gastro intestinal panel resulted in the detection of enterovirus /rhinovirus, adenovirus, norovirus, adenovirus 40/41 and different bacteria.
For the detection of SARS-CoV-2 variants, a 550bp amplicon in the RBD region of the Sgene was sequenced with Sanger and Illumina. This method identifies combinations of mutations to distinguish different variants. Seven of the nineteen samples could be amplified for sequencing by this method. These samples all contained known variants of BA4/BA5, BA4.6/BF7 and XBB. A complete genome could be generated with an Illumina sequencing method with two of these samples. These samples were both identified as BA.4/5 by RBD sequencing. By analyzing the whole genome data with the Freya tool, the samples were found to be specific BA.5 derivatives DY.4 (BA.5.2.48.4) and DY.2 (BA.5.2.48.2).

CONCLUSIONS

In this pilot project we have seen that it is possible to detect SARS-CoV-2 and its variants. The method can be used to provide valuable information coming from areas where SARS-CoV-2 genomic surveillance data are scarce. It can also be used for detection of other diseases like MERS, ebolavirus, Marburg and poliovirus, which can all be detected in fecal samples. This new way of screening is already implemented by the CDC in the USA and can easily be used in Europe for screening flights from high-risk areas.

OP 035

One Health

WAS A PIG VIRUS RESPONSIBLE FOR THE DEATH OF THE FIRST PIG-TO-HUMAN HEART TRANSPLANT RECIPIENT?

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BACKGROUND-AIM

The first pig-to-human heart transplant was conducted in Maryland,USA in January 2022. Whilst the transplanted organ initially demonstrated good acceptance and function, the recipient died 2 months post transplant. Prior to the recipients death, porcine cytomegalovirus was detected in the blood of the recipient. Post-mortem samples from the donor animal were screened again for porcine cytomegalovirus and tested positive. The aim of this study was to evaluate if porcine cytomegalovirus was a contributing factor to the death of the first pig-to-human heart transplant.

METHODS

Samples collect from the recipient were tested for evidence of porcine cytomegalovirus infection vs microchimerism associated DNaemia. Other possible factors contributing to the patient's death were also investigated.

RESULTS

Both porcine cytomegalovirus and porcine DNA were detected, at varying levels, in all samples tested. However, viral mRNA was only detected in the xenograft. Histological investigations did not support porcine cytomegalovirus infection of the recipient organs. However, high serum anti-pig antibodies were detected in the recipient.

CONCLUSIONS

Evidence of antibody-mediated rejection (AMR) was found despite the use of a gene-modified donor. IVIG bound strongly to donor endothelium, possibly causing recipient immune activation. Finally, reactivation and replication of latent porcine cytomegalovirus in the xenograft possibly initiated a destructive inflammatory response.

OP 036

Respiratory viruses

EPIDEMIOLOGY, CLINICAL PRESENTATION AND WHOLE GENOME SEQUENCING OF HUMAN RESPIRATORY SYNCYTIAL VIRUS IN THE 2022/2023 WINTER SEASON

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BACKGROUND-AIM

Human Respiratory syncytial virus (hRSV) is the most common cause of severe respiratory infection in children. The next-generation sequencing (NGS) plays an important role in pathogens surveillance. In this context the whole genome sequencing of hRSV has become a straightforward procedure to trace viral evolution. The aims of this study were to:
I) Develop a protocol for the amplification and sequencing by NGS of whole genome of hRSV-A/B
II) Asses the clinical manifestations associated to hRSV infections
III) Estimate the rate of hRSV type circulation
IV) Analyze the genetic signature characteristic of circulating strains

METHODS

A total of 725 respiratory samples were collected from 645 pediatric patients between Nov 2022 and Mar 2023 and tested with a panel of real-time RT-PCRs for respiratory viruses at Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia. A typing for hRSV-A and HRSV-B was performed with specific real-time RT-PCR on hRSV-positive samples. A sets of primers were newly designed for the amplification of complete genome. hRSV-A and hRSV-B genomes were divided into three segments and three overlapping RT-PCR were developed. Amplicons were used to prepare genomic libraries and sequencing was performed through Illumina technology. The obtained paired sequences were analyzed with the bioinformatic platform INSaFLU, consensus sequences were included in phylogenetic analysis.

RESULTS

A total of 143 patients (22.2% of total) were hRSV-positive, the median age was 1year, 58.7% were males. Among the 143 patients, 90 (62.9%) were hospitalized with respiratory symptoms and patient records were collected: 57/90 (63.3%) developed bronchiolitis; in 53 out of 90 (58.9%) oxygen therapy was required due to respiratory distress and 9/90 (10.0%) were admitted to intensive care unit. Real time-PCR assays were performed on 90/143 (62.9%), detecting: 19 hRSV A and 71 hRSV B. Whole genome amplification was obtained for 40/90 samples (44.4%). WGS, was successful for 36/40 (90.0%) positive samples.

CONCLUSIONS

hRSV is a significant pathogen for public health, and thus we need to be ready for molecular epidemiology surveillance. Based on the obtained data, our WGS protocol is robust and reproducible, resulting in an helpful procedure for tracing the evolution of hRSV.

OP 037

Respiratory viruses

HERPES SIMPLEX VIRUS IS AN IMPORTANT LUNG PATHOGEN IN CRITICALLY ILL PATIENTS – TEN YEARS EXPERIENCE

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BACKGROUND-AIM

Herpes simplex virus (HSV) 1 and 2 are known human herpesviruses causing the skin and mucous lesions and neuroinfections and rarely the organ complications (e.g. hepatitis and pneumonitis). We analysed the data from patients (pts) hospitalised at ICUs from Dept. of Anaesthesiology, Resuscitation and Intensive Care Medicine, Dept. of Pneumology and 3rd Dept. of Surgery (performing the lung transplant program) and tested for presence of HSV in lower respiratory tract samples (LRTs) and whole blood samples (WBs) due to severe damage of the lung tissue (e.g. mechanical chest trauma at the car accident and severe Influenza or SARS-CoV-2 infection etc.).

METHODS

Between 2012 and 2022, we have obtained 2682 LRTs (BALs, endotracheal tube aspirates and sputum) and 2614 WBs from 1953 pts (735 women and 1217 men, median age at first testing was 60.4 yrs. range 0.04-94.85 yrs.). Extraction of nucleic acid was performed by Qiagen Blood Mini kits, QIAsymphony DSP DNA Mini Kit and MoBio extraction kit and Qiagen QIAamp Viral RNA Mini Kit for LRTs. HSV was quantified by in house RQ-PCR assay.

RESULTS

In total, HSV was detected in 461 samples (378 LRTs and 83 WBs) from 281 pts (14.4%). There were 254 pts positive in LRTs (18% of tested in LRTs) with median of 6,53E+04 HSV copies/ml (range 7.14E+04-1,68E+10) and in WBs from 69 pts (4.7% of pts tested in WBs; median 2.0E+03 HSV copies/ml; range 5.0E+02-3.62E+07). In total, proportion of positive samples varied between 2012 and 2022 (18.5%,18.0%,13.2%,13.5%,10.1%,11.6%,25.0%,14.3%,25.9% (2020), 9.7%,12.4%). HSV was detected in only 3.6% of the tested WBs (range 0.6%-12.8%), but in approx. 16.6% (range 10.5%-44%) of LRTs; no HSV-2 was detected. High HSV quantity in LRTs likely associated with HSV pneumonia (above 1.0E+05 copies/ml) was observed in 118 pts (8.36% of pts with tested LTRs) and acyclovir therapy was started; usually with good clinical response. Such approach likely improved survival at the COVID-19 ICU (approx. about 20% comparing the similar units).

CONCLUSIONS

HSV is important lung pathogen which should be tested in ICU patients and subsequential aimed virostatic therapy likely improves the survival of critically ill patients.

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OP 038

Respiratory viruses

MONITORING OF NASAL TYPE I IFN RESPONSE DURING VIRAL RESPIRATORY INFECTIONS: RESPIFERON STUDY

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BACKGROUND-AIM

With millions of deaths worldwide, the COVID-19 pandemic showed that respiratory viral infections (RVI) are a major public health threat. In this context, we need to have the tools to be prepared for the next epidemic events, with rapid tests to help triage patients. Current methods for diagnosing RVI are based on genomic detection (mostly qualitative RT-PCR) with no consensus whether the infection is actually active or not and if the patients is contagious. Viral culture is one of the only way to determine whether the infection is active, but it is a time-consuming technique. An interesting lead to help determine if the infection is active is the type I interferon (IFN-I) response but IFN-I measurement assays are expensive, and IFN levels are difficult to detect due to low circulating levels. In this work we explored interferon-stimulated genes (ISGs) expression during viral infection reflecting IFN-I production.

METHODS

This study included 481 patients hospitalized in Lyon between November 2022 and May 2023. The cohort is divided into two groups: non-infected (n=46) and infected (n=435) based on the result of their nasopharyngeal BioFire® FilmArray® RP2.1plus pouch test result (detecting 22 pathogens). This project tested the BioFire® FilmArray® IFN pouch prototype (FR, Lyon), detecting 6 ISGs and calculating an IFN score from the ratio of the expression of ISGs between each patient sample and healthy volunteer samples. This score was then compared with RT-PCR, RT-qPCR results for all pathogens and also to viral culture results for SARS-CoV-2, Influenza A and B and RSV.

RESULTS

Results showed that a higher IFN score was significantly associated with the infected status (AUC [95% CI]: 0.92 [0.889; 0.9434]). An increase in nasal IFN-I score was individually observed for each RVI studied ($p<0.001$). Higher IFN score was also associated to positive viral culture (AUC [95% CI]: 0.83 [0.7835; 0.8864]).

CONCLUSIONS

Nasal IFN-I response reflects active respiratory viral infection. This assay is easier and faster to perform than viral culture, and can add the information of active infection compared to RT-PCR assays. Altogether, the monitoring of IFN nasal response can offer new perspectives to improve management of patients with respiratory infections and allow optimizing an isolation strategy.

OP 039

Respiratory viruses

PREVALENCE OF VIRAL RESPIRATORY PATHOGENS IN DECEASED PERSONS: A MULTIPLEX QPCR-BASED STUDY COVERING 16 VIRUSES IN >7500 POST-MORTEM SAMPLES

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BACKGROUND-AIM

The SARS-CoV-2 pandemic demonstrated the relevance and risks posed by respiratory viruses, but also greatly expanded interest in viral evolution, transmission patterns and efficacy of infection control measures. Effective surveillance and control is based on the capacity for reliable and timely testing at a large scale, which likewise improved substantially during the pandemic. Whilst much data on respiratory pathogens has been collected on living patients during the pandemic, their prevalence in deceased persons has been considerably less studied.
Aim: Respiratory swabs from decedents in the Hamburg metropolitan region were analyzed for the presence of 16 different viral respiratory pathogens by multiplex qPCR.

METHODS

Respiratory swabs (UTM, MANTACC) were obtained from cadavers examined at the Institute of Legal Medicine and two crematoria. A newly developed multiplex-PCR assay was applied for simultaneous detection of 16 different viral targets (SARS-CoV-2, Influenza A/B, RSV, Rhino-/Enterovirus, Bocavirus, hMPV, Parainfluenzavirus (type 1, 2, 3,4), Adenovirus, hCoV-NL63, hCoV-HKU-1, hCoV-229E, hCoV-OC43) on an automated system (Roche Cobas 5800/6800/8800).

RESULTS

From January 2022 to December 2022, swabs of 7.533 decedents were examined, representing about 35% of the death population in that area (n=4.369 (58%) males, n=3.158 (42%) females). Median age was 76 years with males younger than females (72 years vs 80 years).
Overall positivity rate for repiratory viruses was high with pathogens detected in 1592 (21%) deceased. SARS-CoV-2 was predominantly detected (n=980 (13%)), while positivity rate for all other pathogens was low (Rhino-Enterovirus 2%, Bocavirus 2%, RSV 1%, all others <1%). At the end of the evaluation period we observe a higher overall positivity rate (31% in winter 2022) and increased detection of other pathogens (e.g. FluA and RSV (8% versus 3% positivity rate)) beside SARS-CoV-2 (16% positivity rate in winter 2022).

CONCLUSIONS

Our study provides evidence of a high prevalence of respiratory viruses in deceased persons. Their contribution to mortality should be further investigated.

OP 040

Viral infections in pregnancy

DIAGNOSTIC PERFORMANCE OF DRIED BLOOD SPOT (DBS) MOLECULAR TEST FOR DETECTING CONGENITAL CMV (CCMV) INFECTION IN NEONATES BORN TO WOMEN WITH NON-PRIMARY CMV INFECTION

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BACKGROUND-AIM

To assess the diagnostic performance of Dried Blood Spot (DBS) real-time polymerase chain reaction (PCR) for recognising congenital CMV (cCMV) infection in neonates born to women with non-primary CMV infection compared to conventional reference standard based on saliva sample for initial detection and urine sample for confirmation of congenital infection

METHODS

This study is nested to the Congenital Human CMV infection in Lombardy (CHILd) study, including 9,661 neonates born to 9,503 women with non-primary CMV infection. CMV-DNA was identified in the saliva of 45 newborns and a cCMV infection was confirmed by urine DNA testing in 17 of them. We analysed 31 out of 45 (68.9%) DBS collected from newborns with CMV-DNA positive saliva: of these 12 were from confirmed cCMV. We also analysed 35 DBS collected from newborns with CMV-DNA negative saliva. All DBS were collected within 10 days from birth. DNA was extracted by thermal shock manual process from three 3-mm punches of DBS; the eluates were used directly for in house real-time PCR targeting CMV immediate early region (nt. 379-484). The limit of detection of real-time PCR was 0.5 copies CMV/µl. Each specimen (i.e. 3-mm punches) was tested in triplicate; DBS was considered CMV-positive if at least 1 out of 3 punches was positive at least twice. Human gene ribonuclease P was amplified to monitor DNA extraction

RESULTS

Sensitivity of the DBS test was 83.3% (10/12), specificity was 100% (54/54), positive predictive value (PPV) and negative predictive value (NPV) were 100% (10/10) and 96.4% (54/56), respectively. Thus, the concordance between DBS test and the reference standard method was 97% (64/66)

CONCLUSIONS

The diagnostic performance of DBS molecular test for detecting cCMV was satisfactory. The main strength of this study is that involved exclusively neonates born to women with non-primary CMV infection that should result in a low CMV load in newborns blood; unfortunately, we could not retrieve all DBS from the confirmed cCMV cases. As molecular methods continue to evolve, DBS-based tests should be considered as a promising approach to be included in future cCMV screening algorithms

OP 041

Viral infections in pregnancy

EVIDENCE OF INCREASE RISK OF SEVERE SARS-COV-2 INFECTION AMONG PREGNANT WOMEN WITH MALARIA CO-INFECTION

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BACKGROUND-AIM

There is possibility of malaria and COVID-19 co-infection especially among people living in LMICs, particularly in Africa, courtesy of malaria endemicity in the regions and global spread of COVID-19. Pregnant women are vulnerable to infections, and will be at a higher risk of malaria and COVID-19 co-infection. Herein we aim to study risk factors for severe infection among pregnant women infected with both SAR-CoV-2 and Malaria.

METHODS

Two nasopharyngeal swabs (NS) of the 400 participants were taken. One was used to test for SARS-COV2 antigen using Panbio™ COVID-19 antigen Rapid Test and the other was for RT-PCR. Blood sample was collected into EDTA sample bottle, smear were made, stained with Geimsa stain and microscopically examined for malaria parasite, same was used for malaria PCR. Plasma was used to test for COVID-19 seroprevalence using Standard Q COVID-19 IgG/IgM and disease severity markers (ferritin and D-dimer) using Electrochemiluminescence (ECL) immunoassay.

RESULTS

The result showed that 45(11.3%) of the subject are positive for COVID-19 using RT PCR. 29(7.5%) with rapid antigen kit and 33% for IgG/IgM. For malaria 104(26.0%) of the subject had malaria and co-infection was detected in 33 participants (8.25%). The mean serum concentration of D-dimer and ferritin among subject with malaria-COVID-19 co-infection was 839.09 ± 137.21 and 167.81 ± 17.17 respectively. There was significant increase of means of the markers concentration in subjects with co-infection than in those with mono-infections and negatives (p-value of <0.001*). The markers concentration increases with increase in gestational age (p-value of <0.001*)

CONCLUSIONS

We observed marked increase in the concentration of the disease severity makers in pregnant subjects who had COVID-19 and malaria co-infection compared to subjects having mono infection of both, and these increases with the increase in the gestational age of the subject. This is alarming as the fear of bad pregnancy outcome might be expected in these case. This warrant close monitoring of pregnant women who had COVID-19 and malaria at any time during their pregnancy especially in LMIC and malaria endemic region.

OP 042

Viral infections in pregnancy

EXPRESSION PATTERNS OF CELLULAR RECEPTORS OF THE CYTOMEGALOVIRUS IN THE FETAL HUMAN INNER EAR

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BACKGROUND-AIM

Congenital cytomegalovirus (CMV) can cause sensorineural hearing loss (SNHL) as well as vestibular impairment. However, the pathogenesis remains elusive. In order to gain understanding in the pathogenic mechanism, it is important to know which human fetal inner ear cell types are potential targets to CMV infection.
CMV is known to have a wide tropism. The virus enters the human cell using several glycoproteins among which are the pentameric complex, the trimeric complex, and glycoprotein B. The platelet-derived growth factor alpha (PDGFR〈), neuropilin-2 (NRP2) and endothelial growth factor receptor (EGFR) have been identified as receptors for these viral glycoproteins. However, it is unknown if and at what fetal stage these proteins are expressed in the developing human inner ear.

METHODS

Fetal human inner ears from both the first (N=5) and second trimester (N=6) were obtained by means of elective abortion and fixed directly upon collection. Paraffin sections were immunostained for NRP2, PDGFR〈, and EGFR and counterstained for relevant inner ear cell types. Single nuclei RNA sequencing on fetal inner ear tissue (7 and 9 weeks fetal age, N=2) was used to verify these data.

RESULTS

Immunofluorescent staining showed that NRP2 was expressed in resident macrophages and, in a lesser degree, in the mesenchyme. EGFR was expressed in many cell types such as mesenchymal cells, epithelial cells, spiral ganglion cells and stereocilia in the vestibular organs. PDGFR〈 was also expressed in the mesenchyme, but not always co-expressed with EGFR. All CMV entry proteins were detected in tissue from the first and second trimester. RNA-sequencing data showed a similar expression pattern.

CONCLUSIONS

CMV host factor receptors NRP2, PDGFR〈 and EGFR are already expressed during the first trimester, both in the cochlea and vestibular organs. The proteins are co-expressed in the mesenchymal cells and resident macrophages, which makes these cells potential targets for CMV infection in the developing inner ear. In future experiments, this hypothesis will be tested in an inner ear organoid model which is co-cultured with several CMV strains.

OP 043

Viral infections in pregnancy

HCMV GLYCOPROTEIN M HAS 3 DISTINCT GENOTYPES AMONG CLINICAL ISOLATES

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BACKGROUND-AIM

HCMV is the most common cause of congenital infections affecting 0.2-2.2% of all live births. The virus carries various viral-encoded glycoproteins embedded in its envelope which are important for viral attachment, entry into host cells and replication of HCMV. Some of the glycoproteins have polymorphic features resulting in a number of distinct genotypes. Previous studies have proposed an association between particular genotypes and severity of disease. However, the relationship between the major glycoproteins, particularly glycoprotein complex II, and the pathogenicity of the virus are still poorly understood. Glycoprotein complex II comprises glycoprotein M (gM) and N (gN). Glycoprotein M is the most abundant glycoprotein on the virion envelope, and is essential for viral replication. It is involved with gN in viral entry, the fusion of viral and host cell membrane, and viral spread from cell to cell. gM glycoprotein is known to be highly conserved and has not been considered to exist as different genotypes among HCMV strains.

METHODS

We used molecular techniques (PCR, RFLP, Sanger sequencing and phylogenetic analysis) to look at the sequence of the gM gene of HCMV laboratory strains (AD169, Davis, Towne, Toledo and Merlin), from clinical isolates from patients with HCMV disease and in published HCMV sequences taken from GenBank to investigate any consistent variability within the HCMV gM gene.

RESULTS

Three distinct genotypes for gM were identified among lab strains, clinical isolates and among published gM sequences. It is uncertain yet whether these genotypes cause any changes in any function or binding capability of the gene.

CONCLUSIONS

Polymorphisms in glycoprotein M have not previously been considered, presumably because this major envelope glycoprotein has been considered to be highly stable and not polymorphic. However, in our studies we categorised gM into distinct genotypes due to variability in a small region of the genome. These changes could alter the conformational structure of the glycoprotein, potentially altering its function in vivo.

OP 044

Viruses, tumors and immunocompromised hosts

DIAGNOSTIC UTILITY OF CYTOMEGALOVIRUS DNA QUANTITATION IN PATIENTS WITH ULCERATIVE COLITIS

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BACKGROUND-AIM

Human cytomegalovirus (CMV) colitis is an important clinical entity associated with severe complications and high morbidity in ulcerative colitis (UC) patients. The clinical symptoms of UC exacerbation and CMV colitis are pretty similar. The in situ detection of viral markers by immunohistochemical analysis is the gold standard for a specific diagnosis of CMV colitis. The detection of CMV nucleic acid is widely used and easy to perform. However, the value of the quantification of CMV DNA has yet to be established for diagnosis. This study aims to determine the optimal diagnostic value of CMV DNA in colon tissue and plasma samples from UC patients.

METHODS

Eighty-one adult UC patients suspected of CMV colitis between January 2019 and March 2022 were included in this study. All patients were seropositive for CMV at the time of sampling. The CMV DNA was investigated using the Abbott RealTime CMV assay (Abbott Park, Illinois, U.S.A.) with the real-time polymerase chain reaction (Rt-PCR) method in 81 colon tissues and 43 plasma samples. In tissue samples, CMV DNA copies per mg were calculated. CMV markers were investigated by immunohistochemistry (IHC) and hematoxylin and eosin staining (H&E). The optimal diagnostic viral load in tissue and plasma was assessed based on IHC.

RESULTS

CMV-specific staining was detected 9.8% (n=8/81) in tissue samples by IHC and 1.2% (n=1/81) by H&E ($p<0.001$). CMV DNA was detected 63.0% (n=51/81) [median 20 copies/mg (0-160159)] in tissue samples and 58.5% (n=25/43) plasma samples [median 20 copies/mL (0-1937)]. Based on the IHC, the CMV DNA positivity's sensitivity (SN), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) are 100.0%, 41.9%, 15.7%, and 90.1% for tissue, and 100.0%, 48.6%, 24.0%, and 100% for plasma CMV DNA respectively. With ROC analysis, >392 copies/mg (SN; 100%, SP; 83.6%) in tissue and >578 copies/ml (SN; 66.7%, SP; 83.6%) in plasma were found to be an essential utility for diagnosis.

CONCLUSIONS

In this study, CMV DNA positivity in tissue and blood samples has high sensitivity and low specificity for CMV colitis. However, detection of CMV viral load may be helpful in increased specificity. There is no standardised method for quantifying CMV DNA, especially in tissue samples, so each centre should generate its data.

OP 045

Viruses, tumors and immunocompromised hosts

EPSTEIN-BARR VIRUS LMP-1 PEPTIDE VARIANTS AND HOST-ENCODED HLA-E ALLELES ARE NOVEL BIOMARKERS FOR EBV-ASSOCIATED LYMPHOPROLIFERATIVE DISEASES

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BACKGROUND-AIM

Epstein-Barr virus (EBV) reactivations may cause lymphoproliferative diseases, such as EBV+ non-Hodgkin (EBV+nHL) or Hodgkin (EBV+HL) lymphomas in immunocompetent persons, and post-transplantation lymphoproliferative disorders (EBV+PTLD) in immunocompromised transplant recipients. It is, however, still unclear, why EBV reactivations progress to EBV+ lymphoproliferative diseases only in a minority of patients. HLA-E is a highly conserved, non-classical MHC class I molecule, and two allelic variants, the high-expressing HLA-E*0103 and the low-expressing HLA-E*0101 are prevalent. EBV encodes for a highly polymorphic LMP-1-derived peptide, which stabilizes HLA-E on the surface of EBV-infected cells and inhibits EBV-specific cellular immune responses. In our study, we hypothesized that EBV LMP-1-derived peptides and host-encoded HLA-E variants in the individual host are associated with the progression of EBV reactivations toward EBV-associated lymphoproliferative diseases.

METHODS

We genotyped host HLA-E variants and the EBV LMP-1 peptide variants of the infecting EBV strain in a study cohort including healthy controls (N=96), immunocompetent individuals with EBV reactivations (N=96), EBV+nHL (N=25) and EBV+HL (N=38). We also genotyped transplant recipients with symptomatic EBV reactivations without progression to PTLD (N=144) and transplant recipients with EBV+PTLD (N=36).

RESULTS

We could demonstrate that EBV-strains encoding for the specific LMP-1 peptide GGDPHLPTL or GGDPPLPTL variants are significantly over-represented in immunocompetent individuals as well as in transplant recipients with symptomatic EBV reactivations ($p<0.0001$). The further progression to EBV+nHL, EBV+HL, and EBV+PTLD was highly associated with the presence of both peptide-encoding EBV-strains together with the presence of the high-expressing HLA-E*0103/0103 in the host ($p<0.0001$).

CONCLUSIONS

The risk for EBV+nHL, EBV+HL, and EBV+PTLD depends significantly on the EBV LMP-1 peptide variants of the infecting EBV strain, and of the host-encoded HLA-E variants. The identification of the EBV-LMP-1-derived peptides and HLA-E alleles in patients at risk may provide thus biomarkers that predict EBV-associated lymphoproliferative diseases in the individual host.

OP 046

Viruses, tumors and immunocompromised hosts

EXPRESSION OF HUMAN ENDOGENOUS RETROVIRUSES (HERV) IN CLINICAL SPECIMENS OF PATIENTS WITH COLON CANCER

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BACKGROUND-AIM

Human endogenous retroviruses (HERV) are relicts of exogenous retroviral infections, constituting 8% of the human genome. The genomic structure of HERV is composed of four main genes: group-specific antigen (gag), protease (pro), polymerase (pol) and envelope (env). Alteration of HERV expression has been related to several cancers, but researches regarding HERV gene expression in colon cancer are still sporadic. Very few reports investigated the presence of HERV transcripts in plasmatic extracellular vesicles (EV). The aim of the study was to analyze the role of HERV in colon cancer pathogenesis.

METHODS

Fifty-seven Italian patients with advanced-stage colon cancer were enrolled. The expression of HERV-H, -K, -P env gene, and HERV-K pol gene was analysed in the tumor tissues and negative surgical margins and, when possible, in the peripheral blood. The presence of HERV transcripts was evaluated in the EV, isolated from the plasma of 42 patients. The expression of HERV-K Env protein was evaluated in tumor tissue and in negative surgical margins by Western Blot. Associations among clinical characteristics, and HERV gene expression levels were analysed.

RESULTS

HERV-P env was more expressed in blood compared to tumor tissue ($p<0.05$), while HERV-H, -K env, and HERV-K pol expression levels were similar in the clinical specimens. HERV genes were expressed, at low levels, in the plasmatic EV of 19% (-H env), 38% (-K env), 24% (-K pol), and 17% (-P env) tested patients. Preliminary results showed higher expression of Env protein in the tumor tissue than in the negative margins.

CONCLUSIONS

Differentially expression of HERV-P env may play a role in colon cancer. As known, cancer-secreted EV influence the tumor microenvironment and support cancer growth and metastasis: HERV transcripts may be carried in the circulating plasma EV and transferred from one cell to another, favouring cellular transforming mechanisms. On the other hand, in tumor cells, Env protein might promote cell to cell fusion.

OP 047

Viruses, tumors and immunocompromised hosts

UPDATED EPIDEMIOLOGY OF CYTOMEGALOVIRUS RESISTANCE TO LETERMOVIR IN FRANCE

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BACKGROUND-AIM

Letermovir (LTV), a terminase inhibitor, is available in France since September 2018 for prevention of cytomegalovirus (CMV) infection in stem cell recipients (HSCT) at high risk for CMV. However, it has been used in secondary prophylaxis in the same population or as a rescue therapy in various immunocompromised patients for refractory infections in the absence of maribavir.

METHODS

The French National Reference Center (NRC) is in charge of resistance genotyping and has collected all cases of resistance, on the basis of Sanger sequencing of the terminase complex encoding genes UL56, UL89, and UL51 implicated in LTV resistance. We describe the results of LTV resistance survey updated from 2018 to 2022. The French OMEDIT collected all letermovir deliveries from French hospitals in 2020-2021.

RESULTS

Global prevalence of resistance was estimated from OMEDIT results 2020-2021 and French NRC network: among 1694 patients treated by LTV 30 experienced LTV resistance (1,7%).
In the subpopulation of HCT recipients included in the NaViRe Cohort for New Antivirals efficacy and resistance survey (14 centers in France) 3/193patients developed resistance under letermovir prophylaxis (1,5%). This compares to overall resistance in the HCT patients in 2021 (1,22%) reported from genotyping.
Detail of resistance cases 2017-2023: 17/48 patients experienced LTV resistance and are documented to date: 24 (77,4%) HCT recipients, 5(16,1%) SOT (lung 1, kidney 4) , 1 HIV, 1 idiopathic CD4 lymphopenia; 5/31 (13%) had a previous resistance, to GCV. LTV was given as primary prophylaxis for 16 patients (47%), secondary prophylaxis or curative treatment for others. Viral load at resistance was low or medium (3,93+/-0,74 UI/mL). Known mutations were: UL56 C325Y and C325F/R/W conferring absolute resistance (20, 59%), others were R369M/T, L254F, L257I , V231L, V236M, and 2 new mutations R129H and A102V; 5 patients harbored association of mutations: 2 UL51 mutation P91S and A95V associated with UL56 substitutions and no UL89 known mutations. Most of the resistance case occurred under curative treatment.

CONCLUSIONS

LTV Resistance is not infrequent but occurs mainly during curative treatment or secondary prophylaxis. New mutations in pUL51 underlined the need to include all terminase genes in the genotyping assays

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POSTERS

001

Advancements in diagnostics

A COMPARISON OF FIVE ILLUMINA, ION TORRENT, AND NANOPORE SEQUENCING TECHNOLOGY-BASED APPROACHES FOR WHOLE GENOME SEQUENCING OF SARS-COV-2

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BACKGROUND-AIM

Rapid identification of the rise and spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern remains critical for monitoring of the efficacy of diagnostics, therapeutics, vaccines, and control strategies. A wide range of SARS-CoV-2 next-generation sequencing (NGS) methods have been developed over the last years, but cross-sequence technology benchmarking studies are scarce.

METHODS

In the current study, 26 clinical samples were sequenced using five protocols: AmpliSeq SARS-CoV-2 (Illumina), EasySeq RC-PCR SARS-CoV-2 (Illumina/NimaGen), Ion AmpliSeq SARS-CoV-2 (Thermo Fisher), custom primer sets (Oxford Nanopore Technologies, ONT), and capture probe-based viral metagenomics (Roche/Illumina). Studied parameters included genome coverage, depth of coverage, amplicon distribution, and variant calling.

RESULTS

The median SARS-CoV-2 genome coverage of samples with cycle threshold (Ct) values of 30 and lower ranged from 81.6 to 99.8% for, respectively, the ONT protocol and Illumina Ampliseq protocol. Correlation of coverage with PCR Ct-values varied per protocol. Amplicon distribution signatures differed across the methods, with peak differences of up to 4 log10 at disbalanced positions in samples with high viral loads (Ct-values δ 23). Phylogenetic analyses of consensus sequences showed clustering independent of the workflow used. The proportion of SARS-CoV-2 reads in relation to background sequences, as a (cost-)efficiency metric, was highest for the EasySeq protocol. The hands-on time was lowest when using EasySeq and ONT protocols, with the latter additionally having the shortest sequence runtime.

CONCLUSIONS

In conclusion, the studied protocols differed on a variety of the studied metrics. This study provides data that assist laboratories when selecting protocols for their specific setting.

ESCV 2023
POSTERS

002

Advancements in diagnostics

A NEW DEVICE FOR VAGINAL SELF-COLLECTION IS ABLE TO MAINTAIN HPV NUCLEIC ACIDS AT CHALLENGING TEMPERATURES AFTER SAMPLE LONG TERM STORAGE

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BACKGROUND-AIM

Cervical cancer secondary prevention is progressively transitioning from the established PAP test to HPV DNA testing. This transition increased HPV screening coverage in low to middle income Countries, even for remote communities in extreme environmental conditions. In this study we wanted to test whether a sample first collected using new Copan Self-Vaginal Kit and then eluted in 2 different transport media could withstand high temperatures and maintain sample integrity prior testing using different molecular platforms.

METHODS

A simulated vaginal sample was prepared, at different concentrations, resuspending HeLa cells in vaginal matrix. After immersion of the swab tip in the cellular suspension for 5 seconds, the collected sample was transferred into an empty sterile tube, then stored at 50°C until testing. At several timepoints (0, 7, 10, 14, 21, 28 days) 5ml of Copan Mswab® or ThinPrep™ PreservCyt™ (Hologic) were added to elute sample. Then 230 µl and 300 µl aliquots were used for DNA extraction with PrepSEQ™ Express Nucleic Acid Extraction Kit on AutoMate Express™ Nucleic Acid Extraction System (Thermo Fisher Scientific) and STARMag 96 x 4 Universal Cartridge Kit on Nimbus MicroLab Extraction System (Seegene), respectively. Extracted samples were analyzed using the new Allplex™ HPV HR Detection assay (Seegene) on CFX-96™ Real-Time PCR System (Biorad-Seegene).

RESULTS

The data analyzed show an overall stability of the sample resuspended across the different conditions. There are no statistically significant differences between the average Ct values obtained testing the sample resuspended in MSwab and ThinPrep medium at different storage times and amplified after two different extraction protocol. After 28 days, up to 50°C of conservation of a simulated vaginal sample, the variance of the amplification Ct spans around the value of 1 Ct compared to day zero at room temperature.

CONCLUSIONS

Copan Self-Vaginal kit can be used to preserve and transport vaginal sample up to 4 weeks before elution and testing, even if stored at extreme temperatures. The presence of the empty tube allows the elution of the specimen with the medium and the volume more suited to the different molecular analyses available.

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003

Advancements in diagnostics

A NOVEL AND COMPATIBLE TRANSPORT MEDIUM FOR VIRUSES DETECTION IN SIMULATED OR CLINICAL SAMPLES

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BACKGROUND-AIM

In molecular biology, the availability of several viral identification kits with different targets may require different elution media depending on the compatibility with the analytical platform(s) used. The aim of this study is to identify whether a single non-alcohol based medium (MSwab®, Copan) can be used for the identification of DNA and mRNA of Human papillomavirus and RNA and antigens of SARS-CoV-2 in different platforms in order to simplify the laboratory analysis workflow.

METHODS

Simulated vaginal and respiratory matrices were used for assaying either HPV in HeLa cells or a pool of 10 SARS-CoV-2 positive samples, by using the Hologic platform for HPV mRNA detection and Seegene Anyplex kit for the HPV DNA, and Aptima SARS-CoV-2 (Hologic), Allplex (Seegene), Cobas (Roche) and Lumipulse G SARS-CoV-2 Ag kit (Fujirebio), respectively. Furthermore, vaginal or cervical samples were collected from 32 patients and analyzed as above. This unique analytical approach was allowed thanks to the FLOQSwabs® and MSwab system (Copan). A comparison versus UTM medium (Copan) was also performed.

RESULTS

As far as HPV tests, data showed full compatibility of MSwab system with the Hologic platform. The average relative light units (RLU) for samples eluted in MSwab® was 2,016,334 ± 45,184. Considering two different targets assessed, and the different degree of gene transcription, 20/32 clinical samples were positive at both the HPV DNA and mRNA assays, with no interferences or invalid results detected.
Conserning SARS-CoV-2, data showed an intra-assay consistency in the results obtained with MSwab® or UTM tubes. For MSwab® tubes, mean Ct (±SD) values in the Allplex were 23.1 (±0.2) for E gene, 25.1 (±0.5) for RdRP/S genes and 24.2 (±0.3) for N gene; values in the Cobas were 24.4 (±0.1) for ORF1a/b gene and 24.7 (±0.2) for E gene. The mean relative light unit (RLU) (±SD) values in the Aptima were 1128 (±16) for RNA amplicon. The mean N-antigen (±SD) value was 297 (±42) pg/ml for Lumipulse. No interferences or invalid results were obtained.

CONCLUSIONS

The results from the assays summarized above were consistent, showing the possibility to use MSwab® as an alternative stable medium for the detection of both respiratory swab and vaginal/cervical samples with different targets.

004

Advancements in diagnostics

ALINITY M YOU-CREATE LAB-DESIGNED TEST FOR HEPATITIS D VIRUS

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BACKGROUND-AIM

Hepatitis D is a viral infection that causes liver inflammation and damage. Hepatitis D Virus (HDV) infection occurs only in those who are infected with the Hepatitis B Virus and can be observed as acute or chronic infection. About 5%of those with chronic Hepatitis B Virus (HBV) infection also are infected with HDV with chronic HDV infection resulting in a more aggressive and rapid progression of liver disease than HBV infection alone. Lab developed tests (LDTs) play an important role in the diagnosis of infectious diseases, especially during an outbreak or for rare disease for which a regulatory approved test is not available. Current molecular diagnostic platforms are capable of continuous testing of multiple analytes with some allowing users to run LDTs in open-access mode. The Alinity m You-Create feature allows LDTs to be run alongside commercially available Alinity m assays. Here, we optimized and evaluated the performance of the HDV LDT with the Alinity m You-Create feature.

METHODS

HDV primers and probes were adapted from a previously clinically validated HDV LDT run on the Abbott m2000 sp/rt platform. PCR reaction was optimized using the above HDV primers and probe alongside Alinity m master mix reagents. Cycling conditions were optimized to meet the Alinity m instrument requirements to allow the LDT to be run alongside commercially available Alinity m assays. Feasibility was assessed using a positive clinical specimen that was diluted to 1-1,000 IU/mL in normal human plasma.

RESULTS

Alinity m You-Create HDV LDT detected 100% of replicates at concentrations between 1-1,000 IU/mL. HDV LDT assay demonstrated linear performance across this range (r2=0.991).

CONCLUSIONS

This study successful demonstrates the Alinity m You-Create assay implementation of an established LDT for the Alinity m platform. The assay detected the HDV as low as 1 IU/mL and performed in a linear manner and supports the transition to testing of the clinical samples.

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005

Advancements in diagnostics

ASSESSING THE DETECTION CAPABILITY OF MOLECULAR DIAGNOSTICS USING INTERNATIONAL STANDARDS AND QUANTIFIED CONTROL MATERIALS FOR HERPESVIRUSES IN CENTRAL NERVOUS SYSTEMS INFECTIONS

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BACKGROUND-AIM

Laboratory diagnosis of central nervous system (CNS) infections often relies on the use of molecular diagnostic testing in cerebrospinal fluid (CSF) which is a key tool for the diagnosis of viral infections. Herpesviruses are one of the most common groups of pathogens causing viral CNS infections in humans. The determination of Limit of Detection (LoD) for the molecular detection of herpesviruses is an essential step in performance evaluation.

METHODS

WHO International Standards and a quantified control material were obtained for three human herpesviruses. A dilution series of the HSV-1, HSV-2, and VZV materials was prepared in a synthetic cerebrospinal fluid sample matrix. Sample dilutions were tested in 24 replicates with molecular samples-to-answer tests [BioFire® Meningitis/Encephalitis (ME) Panel, Diasorin Simplexa™ R-GENE® kit (Argene, bioMérieux) according to the instructions for use for use. Data were compiled and hit rates. LoD was estimated empirically by the Detected/Not Detected response (hit rate) and also by statistical Probit analysis using the Finney method (Microsoft Excel) or using Minitab Statistical Software (Minitab, LLC; Probit/maximum likelihood estimate method) which includes the 95% confidence interval of the estimated LoD.

RESULTS

Testing standard materials demonstrated that detection of HSV-1, HSV-2, and VZV by all three test kits is comparable within 1.5-fold to 6-fold. Detection by the BioFire® ME Panel is comparable (within 1.5-fold to 4-fold) to the HSV1&2 VZV R-Gene® test kit and is comparable (within 1.5-fold to 3-fold) to the Simplexa™ HSV 1 & 2 Direct and VZV Direct kits. Direct (empirical) LoD estimates based on hit rates are comparable to statistical 95% LoD concentration estimates. Different statistical methods can generate different LoD values from the same data.

CONCLUSIONS

WHO International Standards and quantified commercial control materials allow for more informative comparisons within and between tests relative to LoD reported in non-molecular units (e.g. TCID50/mL). Goodness of fit and confidence intervals are important in understanding the accuracy and error of a statistically-determined LoD for the establishment of the herpesviruses identification using BioFire® ME Panel.

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006

Advancements in diagnostics

AUTOMATED WHOLE BLOOD PRETREATMENT SOLUTION FOR ALTOSTAR® AM16 WORKFLOW

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BACKGROUND-AIM

A higher sample-throughput can be achieved when automation of sample pretreatment is performed. Based on data collected in routine diagnostic laboratory we present an automated solution for the AltoStar® AM16 suitable for the pretreatment of whole blood.
Preparation of whole blood in routine diagnostic usually needs to be done manually before downstream automated purification. This manual step can be error prone and requires elaborate data management steps to ensure sample traceability in the laboratory information system (LIS).

METHODS

Our software solution allows the user to import a sample input file from the LIS for the pretreatment software in the same way as for the routine AltoStar® Workflow. Using the data of the input file the pretreatment will be conducted fully automated by the AltoStar® AM16 instrument. An automatically generated output file serves as a regular input file.

RESULTS

We validated the process with whole blood samples in a routine diagnostic laboratory in Toulouse. The comparability of the performance of the manual whole blood pretreatment and the automated whole blood pretreatment was determined by analyzing the viral load results of CMV and EBV.
More than 95 % of the samples were successfully processed with the pretreatment software in combination with the AltoStar® AM16 and the AltoStar® CMV PCR Kit 1.5 and the AltoStar® EBV PCR Kit 1.5.

CONCLUSIONS

The automated whole blood pretreatment software tool is an easy to use and time efficient application to integrate whole blood pretreatment into the routine diagnostic workflow. Further evaluation will be done.

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007

Advancements in diagnostics

CLINICAL PERFORMANCE EVALUATION OF A CE-IVD DIAGNOSTIC TEST FOR DETECTION OF ALL ENTEROVIRUS SPECIES

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BACKGROUND-AIM

Enteroviruses are associated with a wide spectrum of diseases in humans ranging from minor illnesses such as common colds and hand foot and mouth disease, to life-threatening conditions including viral meningitis, and paralytic poliomyelitis. Because circulating serotypes can cause large disease outbreaks, detection of enterovirus infection is critical to monitor viral spread. The TaqPath™ Menu GeneProof™ Enterovirus PCR Kit is a molecular diagnostic test that detects all enterovirus species including Coxsackievirus, Echovirus, Enterovirus and Poliovirus. The aim of this study was to evaluate the performance of the TaqPath Menu™ GeneProof™ Enterovirus PCR Kit in comparison to other currently available diagnostic assays for the detection of all enterovirus species.

METHODS

Two evaluations were performed. A retrospective study was conducted using 19 deidentified clinical samples (stools, CSF, swab). All samples were tested using the TaqPath Menu GeneProof Enterovirus PCR Kit and CDC Enterovirus VP1 certified method and AusDiagnostics Respiratory Viruses 16-well CE-IVD kit were used as reference methods. Additional 85 samples were further analyzed through the TaqPath Menu GeneProof Enterovirus PCR Kit and various comparator tests.

RESULTS

Of the 11 positive samples by the CDC and AusDiagnostics methods, 11 samples showed a positive result using the TaqPath Menu GeneProof Enterovirus PCR Kit resulting in the clinical sensitivity of 100%. Of the 8 negative samples by the CDC and AusDiagnostics methods, all samples tested negative using the TaqPath Menu GeneProof Enterovirus PCR Kit resulting in the clinical specificity of 100%. Regarding the 85 additional tested samples, the TaqPath Menu GeneProof Enterovirus PCR Kit demonstrated good concordance with the reference assays for detection of enteroviruses with clinical sensitivity and specificity of 100% and 91.67%, respectively. The overall performance comparison of the TaqPath Menu GeneProof Enterovirus PCR Kit and the reference methods between the two studies was 100.00% for clinical sensitivity and 93.75% for clinical specificity.

CONCLUSIONS

The TaqPath™ Menu GeneProof™ Enterovirus PCR Kit is a highly accurate method for detection of enteroviruses.

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Advancements in diagnostics

CLINICAL PERFORMANCE EVALUATION OF A DIAGNOSTIC TEST FOR DETECTION AND DIFFERENTIATION OF HERPES SIMPLEX 1 AND 2 VIRUSES

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BACKGROUND-AIM

Herpes simplex viruses types 1 and 2 (HSV-1/2) cause often recurrent infections in humans mainly affecting the skin, mouth, lips, eyes and genitals. Severe infections including encephalitis, meningitis or disseminated infections in immunocompromised patients can also occur and patients benefit from early administration of antiviral therapy in terms of disease duration and symptom severity. Early diagnosis is therefore important for patient management and prevention of viral transmission which usually occurs through direct contact with infected individuals. In this study we evaluated the clinical performance of the TaqPath™ Menu GeneProof™ Herpes Simplex Virus (HSV-1/2) PCR kit.

METHODS

In total 112 clinical samples collected from individuals undergoing routine diagnostic screening were tested in parallel with the TaqPath™ Menu GeneProof™ HSV-1/2 PCR kit and either the artus HSV-1/2 LC PCR Kit or HSV1&2 VZV R-GENE® test. Positive and negative percent agreement (PPA and NPA) between the TaqPath™ Menu GeneProof™ kit and combined comparators were calculated.

RESULTS

The presence of HSV-1 was detected in 56 samples, while 26 samples were positive for HSV-2 using the TaqPath™ Menu GeneProof™ HSV-1/2 PCR kit. In total 3 samples showed a discordant result between the TaqPath™ Menu GeneProof™ test and the comparator, with all 3 detected as positive with the TaqPath™ Menu GeneProof™ test (2 for HSV-1, 1 for HSV-2). In total 30/112 samples were negative and results were concordant between the different tests. Overall PPA and NPA for the TaqPath™ Menu GeneProof™ test and the comparators were 100% and 90.9%, respectively. The 3 discordant samples were shown to have high Ct values indicating a likely difference in the limit of detection of the CE-IVD tests.

CONCLUSIONS

TaqPath™ Menu GeneProof™ Herpes Simplex Virus (HSV-1/2) PCR kit shows excellent performance for the detection of HSV-1 and HSV-2 infections in clinical samples. With a quick turn-around time of a few hours, this qPCR-based test could be a useful tool in diagnosing HSV-1/2 infections and enabling timely therapeutic decisions, thus potentially improving patient outcomes.

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Advancements in diagnostics

CLINICAL PERFORMANCE EVALUATION OF THE QUANTITATIVE ALINITY M BKV ASSAY

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BACKGROUND-AIM

The BK virus (BKV) is a member of the polyomavirus that reaches seroprevalence over 90% by 4 years of age. BKV primary infection or reactivation increases risk for complications in immunocompromised individuals posing increased risk for developing BKV-associated nephropathy (BKVAN) in kidney transplant recipients and increased risk for BKV-associated hemorrhagic cystitis (BKV-HC) in allogeneic hematopoietic stem cell transplant (HSCT) recipients. High BKV viral load has been reported as predictive factor of risk for BKVAN and BKV-HC. Here, we evaluated the clinical performance of the Alinity m BKV assay.

METHODS

Clinical performance of the Alinity m BKV assay for plasma specimen was compared to Altona RealStar BKV and ELITech Alert MGB BKV ASR run on the Abbott m2000 sp/rt platform using either total nucleic acid (TNA) or DNA extraction protocols. Clinical performance of Alinity m BKV for urine specimens was compared vs ELITech Alert MGB BKV ASR run on the Abbott m2000 sp/rt platform using the TNA extraction protocol.

RESULTS

For plasma specimens, the correlation coefficient and the mean bias between Alinity m BKV and Altona RealStar BKV was 0.970 and -0.47 Log IU/mL; the correlation coefficient and the mean bias between Alinity m BKV and ELITech Alert MGB ASR tested was 0.900 and 0.03 Log IU/mL with specimens extracted using the DNA extraction protocol and 0.963 and -0.54 Log IU/mL with specimens extracted using the TNA extraction protocol. For urine specimens, the correlation coefficient and mean bias between Alinity m BKV and ELITech Alert MGB ASR tested was 0.917 and 0.09 Log IU/mL using the TNA extraction protocol.

CONCLUSIONS

Clinical performance of Alinity m BKV assay was compared to other commercially available BKV assays where the mean bias ranged between 0.03 to -0.54 log IU/mL. The differences in performance could be attributed to the differences in the assay design e.g. target region, sample extraction, cycling parameters, and calibration strategy. Despite these differences, this study demonstrated that the Alinity m BKV assay correlates well with the other comparator assays and supports its utility in transplant patient management.

010

Advancements in diagnostics

CMV-RNA DETECTION AS NEW MARKER IN CMV INFECTION, AFTER HEMATOPOIETIC STEM CELL TRANSPLANTION IN CHILDREN

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BACKGROUND-AIM

Letermovir blocks CMV infection by inhibiting the viral terminase and the virion maturation, without blocking viral-DNA production. Approved in adults, it represents an attractive alternative for CMV-treatment also in children. Effective lab tests for monitoring the drug efficiency are missing, mostly because the CMV-DNA is unable to properly evaluate antiviral efficacy. We present preliminary data on CMV monitoring by a new commercial assay for CMV-RNA detection and quantitation in a cohort of hematopoietic stem cell transplanted children after antiviral treatments

METHODS

Between July-to-November 2022, 53 children were treated with Letermovir after HSCT at the Bambino Gesù Children's Hospital. Thirty-six received primary or secondary prophylaxis, 17 pre-emptive or treatment therapy (median dosage 240 mg/mq, range 120-480). CMV-DNAemia was evaluated twice a week (artus® CMV-TM-PCR_Qiagen_ Hilden, Germany). In 30/53, CMV-RNA was also investigated in plasma samples using CMV-RNA ELITe MGB® (Kit-ELITe_InGenius System: ELITechGroup).

RESULTS

61 samples, referred to 30 patients (median age 10, IQR 1-21 years), were collected to perform simultaneously CMV-DNAemia and CMV-RNAemia. In 54/61 samples (88.5%) CMV-DNA was persistently present (median 848 copies/mL, IQR 314-3995), while only in 16/61 (26.2%) CMV-RNA was detected (median 23 copies/mL, IQR 6-150). When CMV-DNAemia was quantified <1000 copies/mL (26/54 samples including 11 samples pre-treated with DNAsi), RNA-CMV resulted not detected or below 30 copies/mL. Under pre-emptive therapy/treatment of infection, all patients achieved CMV-DNA negativization in blood within 20 days.

CONCLUSIONS

Despite the presence of positive CMV-DNA, the clinical evolution was compatible with the absence of CMV activity thus suggesting that the undetectability of CMV-RNA is driven by the infection evolution and not by the lack in the method sensitivity.
The CMV-RNA may represent an accurate marker to monitor clinical antiviral efficacy, more than CMV-DNA.

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Advancements in diagnostics

COMPARISON OF PBMC EXTRACTION METHODS TO IMPROVE SARS-COV-2 T CELL RESEARCH

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BACKGROUND-AIM

It has become apparent that T cells play a dominant role in immune protection against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). There is a requirement of developing reliable methods, which are easy to use and allow upscaling, to measure the specific T cell response. One purpose of this study was to compare density gradient centrifugation with two automatized methods of magnetic-bead purification for the extraction of peripheral blood mononuclear cells (PBMC) regarding yield and purification quality. The second goal was to evaluate the effect on sensitivity of each extraction method in a SARS-CoV-2 Interferon-gamma Release Assay (IGRA).

METHODS

Employees of a medical laboratory with one, two or three booster vaccinations agreed to blood collection 2 weeks to 6 months after vaccination or infection. The gradient density centrifugation was performed using Leucosep tubes pre-filled with Ficoll-Paque® Plus. The magnetic-bead extractions were done with the T-Cell SelectTM kit by Oxford Immunotec in the AllSheng Auto-Pure20B (AP-20B) and the Thermo ScientificTM KingFisher Flex. The T-SPOT®Discovery SARS-CoV-2 kit by Oxford Immunotec was used for the IGRA.

RESULTS

The PBMC extraction by binding to magnetic beads resulted in higher white blood cell counts per volume and lower contamination of red blood cells than the extraction by density gradient centrifugation (n = 43 – 49). In the subsequent IGRA, the magnetic-bead methods also led to more spots in the panels, which formed where Interferon gamma had been released by sensitized T cells. Additionally, we were able to show that a smaller volume of the bead reagent will increase the number of spots further (n = 32).

CONCLUSIONS

For SARS-CoV-2 related T cell research, automatized magnetic-bead mediated PBMC extraction with the T-Cell SelectTM kit is a viable alternative to the often-used density gradient centrifugation. It enhances the yield and purification quality as well as the sensitivity in the T-SPOT®Discovery SARS-CoV-2 assay.

012

Advancements in diagnostics

COMPARISON OF THE PERFORMANCE OF TWO TARGETED METAGENOMIC VIRUS CAPTURE PROBE-BASED METHODS USING SYNTHETIC VIRAL SEQUENCES AND CLINICAL SAMPLES.

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BACKGROUND-AIM

Introduction: Viral metagenomic sequencing enables pathogen-agnostic detection of viruses in clinical samples, however accurate sensitivity and specificity remains a challenge. In this study, the performance of two virus capture probe-based methods was compared.

METHODS

Method: The following hybridization capture methods were evaluated: the SeqCap EZ HyperCap workflow (Roche) and the Twist Comprehensive Viral Research Panel. In order to mimic the complexity of clinical samples while reducing the number of environmental sequences, a panel was prepared consisting of synthetic respiratory viral sequences (SARS-CoV-2, influenza A virus, measles, enterovirus D68, bocavirus) in varying concentrations, and combined with 90-99% human cell free DNA (cfDNA) background sequences. In addition, ATCC Virome Virus Mix of human mastadenovirus F, human herpesvirus 5, human respiratory syncytial virus, influenza B virus , reovirus 3, and zika virus, each 16.7%, was analyzed and eight clinical samples with viral loads between 500 and 50,000 IU/ml of adenovirus, Epstein Barr virus and hepatis B virus were included. Sequencing was performed on the NovaSeq6000 (Illumina), and metagenomic tool Genome Detective was used for assembly and classification of sequence reads.

RESULTS

Results: The virus capture probe-based methods showed comparable sensitivity for detection of the synthesized sequences over the different concentrations with varying proportions of human background sequences. The clinical samples were challenging with regard to some of the lower viral loads. Additional findings were detected by both hybridization capture methods and in both the synthetic and clinical samples.

CONCLUSIONS

Conclusion: Though the SeqCap EZ HyperCap workflow and Twist Comprehensive Viral Research Panel are composed of unidentical probe sets, they performed corresponding in this comparison, and are applicable for the detection of a broad range of RNA and DNA viruses in clinical samples.

013

Advancements in diagnostics

COMPARISON OF TWO POINT-OF-CARE RESPIRATORY PANELS FOR THE DETECTION OF INFLUENZA A/B VIRUS

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BACKGROUND-AIM

Rapid and accurate diagnostics of patients with suspected seasonal influenza or pathogens of the upper respiratory tract is crucial. The current study aimed to evaluate the diagnostic performance of the QIAstat-Dx Respiratory Panel and the BioFire FilmArray Respiratory Panel 2 plus regarding the influenza A/B virus compared to the AlereTM i Influenza A&B as the comparator method.

METHODS

We compared the performance of two syndromic testing methodologies (QIAstat-Dx RP, BioFire RP2plus) against the AlereTM i as the comparator method. Totally, 97 swab samples were included from patients with symptoms of acute respiratory infection admitted in the hospitals of the wider region of Crete, Greece.

RESULTS

The Positive Percent Agreement (PPA) of the BioFire RP2plus was 100% (95% CI 87.66%-100%), while the Negative Percent Agreement (NPA) was estimated at 91.3% (95% CI 82.03%–96.74%). This method produced no invalid results. For QIAstat-Dx RP the PPA was 89.29% (95% CI 71.77%-97.73%), while the NPA was 91.3% (95% CI 82.03%-96.74%, 63/69). The BioFire RP2plus managed to determine the subtype in more samples than the QIAstat-Dx RP.

CONCLUSIONS

Given the co-circulation of several respiratory pathogens during the flu season and considering the relatively frequent incidences of co-infections in hospitalised patients with a past medical history, syndromic testing proves to be a comprehensive laboratory method, delivering timely results which directly influence medical decision-making.Both panels can be valuable tools for clinicians, since they both display high sensitivity and specificity. We report a slightly better performance for BioFire RP2plus, since it produced no invalid results.

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Advancements in diagnostics

DEFINING HSV-IGG SEROPOSITIVITY - NOT AS EASY AS YOU THOUGHT

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BACKGROUND-AIM

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are common infections of the skin and genitals. Several patient groups are at risk for serious sequelae especially when primary infection occurs. It is therefore important to define HSV serostatus in order to estimate the risk and establish prophylactic measures. For measurement of HSV-1/2-IgG many commercial assay systems are on the market, but only few data are available on test accuracy of these assays. We compared several assays for the detection of HSV-IgG in a group of medical students without knowledge of HSV history.

METHODS

317 sera were measured using a HSV-1/2 EIA based on whole virus lysate (Serion), DiaSorin LIAISON HSV1/2 CLIA based on glycoprotein G; and Euroimmun typespecific assays based on glycoprotein C1 and C2 respectively. Discrepant samples and samples positive by Euroimmun HSV-2-IgG were measured in three different HSV1 and 2 immunoblot assays (Mikrogen, Virotech and Euroimmun). A group of samples with remaining discrepancies was finally measured using an in house assay for the detection of Fc gamma receptor activating antibodies against HSV1 or HSV2.

RESULTS

256 of 317 samples showed concordant results, 91 were negative and 165 were positive for HSV-IgG. 144 samples were HSV-IgG positive using Serion ELISA but only 107 by DiaSorin LIAISON CLIA and 101 by Euroimmun HSV-1 and/or HSV-2-ELISA. Discrepant samples, samples with greyzone results as well as HSV-2-IgG positive samples were further analysed. Using three different immunoblot assays 31 samples showed discrepant results. 14, 7 and 6 positive samples were untypable by Mikrogen, Euroimmun and Virotech assays. Using the Fc gamma receptor activation assay most samples were clearly negative or positive for HSV1 or HSV2. Four samples remained unresolved. Two of five HSV2 positive samples were HSV-2 negative in Virotech Lineassay

CONCLUSIONS

Assays based on glycoprotein G/C as antigen have low sensitivity and are therefore not suitable for seroprevalence studies and definition of HSV serostatus in patients.. Without a true gold standard for the definition of HSV-IgG positivity several samples remain unresolved. Manufacturers and specialized laboratories should try to establish HSV-IgG assays with better sensitivity and specificity.

015

Advancements in diagnostics

DETECTION OF CARDIOTROPIC VIRUSES AND THEIR TRANSCRIPTS IN ENDOMYOCARDIAL BIOPSIES OF PATIENTS WITH UNEXPLAINED HEART FAILURE VIA TARGETED NEXT GENERATION SEQUENCING

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BACKGROUND-AIM

Heart failure can be caused by viral infection of the heart muscle. Differentiating between non-infectious and infectious etiologies is paramount for effective treatment and therefore patient outcome. As therapies for different viruses are highly specific, reliable identification of the causative agent is necessary. We aimed to establish a novel diagnostic method to detect known and suspected/novel cardiotropic viruses and their gene transcripts from endomyocardial biopsies (EMBs) of heart failure cases using a hybridization capture and next generation sequencing approach.

METHODS

Sequencing libraries were prepared from EMB-extracted DNA/RNA. Targeted enrichment via hybridization capture of virus-specific libraries was performed using a custom RNA probe set covering 84 known and suspected cardiotropic viral species. Libraries were sequenced on the MiSeq platform in paired-end mode (2x150bp). Trimmomatic was used for adapter and quality trimming. Taxonomic classification was achieved using Kraken2. Genomic mapping of transcript-specific reads was performed via HiSat2 and analysed using Geneious Prime.

RESULTS

Targeted enrichment allowed detection of Parvovirus B19 (B19V), Epstein Barr virus (EBV), recombinant Enterovirus, and Influenza A virus genomes in EMBs of heart failure patients without prior sequence-specific amplification. Two rounds of enrichment increased percentage of EBV-specific reads 44-fold, compared to one round (15.02% vs 0.34%). Virus-specific transcript reads were detected for both B19V and EBV, with the latter showing differing patterns of latent and lytic genes, with presence of lytic genes corresponding with increased inflammatory infiltrates in the heart muscle.

CONCLUSIONS

The presented method permits the unbiased detection of known and suspected cardiotropic viruses from patient EMBs. Detection of active/lytic viral transcription necessitates a paradigm shift in treatment approaches for these patients from immunosuppression to antivirals in order to not exascerbate infection. Our work highlights the usefulness of comprehensive pathogen detection in cases of unexplained heart failure using sensitive and adaptable enrichment strategies.

016

Advancements in diagnostics

DEVELOPMENT OF A NOVEL GRAPHENE FIELD-EFFECT TRANSISTOR BASED ELECTRONIC BIOSENSOR FOR RAPID, HIGHLY SENSITIVE SARS-COV-2 RNA DETECTION AND DETERMINATION OF INFECTIVITY

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BACKGROUND-AIM

The need for innovative diagnostic procedures to address infectiological questions has been highlighted by the COVID-19 pandemic. Graphene field-effect transistor (gFET) technology is known for use in the biosensor field, with multiple potential advantages compared to PCR (e.g. 30min time to result, highly scalable direct analysis, small footprint). The aims of the FWF funded collaborative COSENZA project (FWF P35103-B) are to improve this technology and develop a fast one step multiplexing diagnostic test procedure for the (I) detection of viral nucleic acids (SARS-CoV-2 RNA), (II) discrimination between infectious and non-infectious samples and (III) identification of variant specific mutations. Here we present the first results of COSENZA for aims (I) and (II).

METHODS

244 nasopharyngeal swab samples have been tested comparatively to RT-PCR (Roche Cobas 6800) so far. Reverse SARS-CoV-2 E-gene DNA was immobilized on the sensor surface to capture SARS-CoV-2 RNA and effects of specific binding in the presence of SARS-CoV-2 RNA were observed as a Dirac point (V_{Dirac}) shift in the transfer characteristics. Experiments testing for nucleocapsid protein (NP), which indicates presence of replicative virus, were conducted to distinguish infectious from non-infectious samples. Monoclonal antibodies capturing SARS-CoV-2 NP were used on samples from patients at different time points throughout their infection. Change in the slope of the transfer characteristic, i.e. current alteration, correlated with timepoints (beginning and end of infection). Statistical analyses were done in MedCalc and OriginLab.

RESULTS

For E-gene, V_{Dirac} measurements correlated with the RT-PCR Ct values in SARS-CoV-2 positive samples. For Ct values of 12-19, 20-29, and 30-39, the gFET read-outs were 0.21 V (±0.04V) with n=13, 0.18 V (±0.06V) with n=10, and 0.06 V (±0.03V) with n=9, respectively, indicating a sensitivity and specificity comparable to RT-PCR. For 160 negative samples (tested in pools of 5), the gFET read-out was 0.03 V (±0.01V). NP results (n=31) showed a decrease in infectivity over time, consistent with cell culture data of the patients.

CONCLUSIONS

gFET-based biosensing is a promising technique for rapid RNA detection as well as determining infectivity.

017

Advancements in diagnostics

DIAGNOSTIC LABORATORIES AS SENTINELS FOR EPIDEMICS USING CROSS-SECTIONAL VIRAL LOAD DISTRIBUTIONS.

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BACKGROUND-AIM

Even as COVID-19 transitions from the pandemic to the endemic phase, new emerging SARS-CoV-2 variants could continue to fuel COVID-19 surges. Early detection of impending waves in the community is critical for public health preparedness and appropriate responses. We recently demonstrated in the United States that diagnostic laboratories can use cross-sectional cycle threshold (Ct) values obtained from PCR tests (Ct-based model) for early detection of COVID-19 and influenza A waves in the community. In this study, we aimed to expand our work to other countries and regions.

METHODS

Cross-sectional Ct values from RT-PCR data generated daily at Pharmgenetix GmbH between May 2020 and January 2023 using the TaqPath™ RT-PCR COVID-19 kit on samples collected from the Salzburg region in Austria, were used to calculate the effective reproductive rate (Rt) and estimate epidemic trajectories. Incidence and Rt estimates from the Ct model were compared to those generated using the positive cases from PCR tests conducted at the Pharmgenetix laboratory (lab-only) and the ones reported by the Salzburg health agency for the entire region. These epidemic dynamics were also tracked through multiple waves of SARS-CoV-2 variants. Pairwise cross-correlation coefficients were estimated between the three trajectories to identify lead times between the laboratory Ct-generated trajectories vs. the case-based trajectories from lab and regional levels.

RESULTS

The Ct-based model detected SARS-CoV-2 waves earlier than case-based trajectories generated in the region. Pairwise cross-correlation showed that Ct-based model predictions preceded general Salzburg region and lab-only cases by 12 days (correlation coefficient = 0.54), and by 10 days (correlation coefficient = 0.65), respectively.

CONCLUSIONS

Ct-based epidemic trajectories could serve as sentinels for early detection of impending COVID-19 waves in specific areas, as new SARS-CoV-2 variants continue to emerge. Taking into account that Ct-values generated by different COVID-19 tests may differ, the publication of RT-PCR Ct values along with the binary test results by global and national public health agencies for cases tested using the same molecular diagnostic test could be valuable for public health responses.

018

Advancements in diagnostics

DISTINGUISHING ACUTE HUMAN BOCAVIRUS 1 RESPIRATORY INFECTION FROM PERSISTENT SHEDDING OF VIRAL DNA

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BACKGROUND-AIM

Human bocaviruses (HBoV) are ssDNA viruses of the Parvoviridae family, first discovered in 2005 in nasopharyngeal samples of children. The virus is known to cause mild to life-threatening respiratory illnesses in children, but also to persist for months after the acute infection, leading to misrepresentations of the true diagnosis. Our aim was to differentiate HBoV1 DNA persistence from acute infections. Our hypothesis was that persisting HBoV1 genomes are in a non-infectious naked DNA form, whereas acutely infecting HBoV1s constitute full virions.

METHODS

We treated clinical samples with the endonuclease Benzonase (2.5 units/µl), to reveal whether or not the viral DNA was protected by a capsid. For this assay, we selected highly characterized samples from acute and past HBoV1 infections; 10 serum, 20 NPA and 10 NPS (with follow-up samples at 2 weeks and 2 months). After a 1-hour nuclease treatment, we performed qPCR on treated and non-treated samples.

RESULTS

The nuclease treatment did not reduce the HBoV1 DNA quantity in serum or respiratory samples from acute HBoV1 infection but did significantly reduce the quantity in samples taken in persistent infection, suggesting that the latter samples comprised naked HBoV1 DNA. Additionally, in the NPS acute and follow-up samples at 2 weeks and 2 months after acute infection, the first two samples had no signification changes in HBoV1 DNA after nuclease treatment, indicating they carried infectious HBoV1 virions; these patients were IgM positive and had IgG of low avidity. Contrary to this, the 2-month samples were still PCR positive for HBoV1 DNA, but became negative after the nuclease treatment, stating that the persisting HBoV1 DNA was in a naked form. The positive virion control was positive, while the naked DNA negative control was negative in all experiments.

CONCLUSIONS

The results obtained provide a proof of concept that nuclease pretreatment prior to PCR can distinguish between acute and persistent HBoV1 respiratory infection. Additionally, our results show that HBoV1 DNA can persist for weeks after infection. Hence, the nuclease pretreatment of serum, NPA and NPS samples could be used in routine diagnosis of HBoV1 infections to discriminate acute infection (virions) from persistent shedding of naked DNA.

019

Advancements in diagnostics

ESTABLISHMENT AND CLINICAL VALIDATION OF A LABORATORY DEVELOPED HEXAPLEX DIGITAL PCR ASSAY FOR ABSOLUTE QUANTIFICATION OF EBV, CMV, VZV, HSV 1, HSV 2 AND BETA-GLOBIN FROM HUMAN TISSUE SAMPLES.

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BACKGROUND-AIM

Reactivation of latent herpesvirus infection can be life-threatening, especially in immunocompromised patients. Viral DNA detection and quantification in tissue samples is challenging, as matrices vary considerably. To overcome this issue, we established and validated a hexaplex PCR assay on a novel digital PCR system (digital LightCycler, Roche) using the high-resolution plates (>100,000 partitions/sample).

METHODS

Analytical LLoD (lower limit of detection; 2-fold, 9 steps, 8 repeats/step) and linearity (10-fold, 3/4 steps, 3 repeats/step) were determined by serial dilution of clinical samples. Tissue biopsies (intestine, lung, brain, liver and skin) were used to evaluate clinical performance. Samples were homogenized in 1 ml H_2O using the Precellys system and nucleic acid extraction was performed on the QiaSymphony platform.

RESULTS

In silico analysis did not reveal any critical oligonucleotide interactions. LLoDs were determined to be 214 cop/ml (95% CI: 143–708 cop/ml), 217 cop/ml (95% CI: 140–566 cop/ml), 162 cop/ml (95% CI: 120–377 cop/ml), 236 cop/ml (95% CI: 162–619 cop/ml) and 214 cop/ml (95% CI: 143–708 cop/ml) for the assays detecting EBV, CMV, VZV, HSV 2 and HSV 1, respectively. Linearity assessment showed slopes ranging from -1.049 to -0.96 and Pearson's correlation coefficients >0.98 for all targets within the tested range (10^3-10^6 cop/ml). Precision was evaluated over the course of three days and showed excellent reproducibility with inter- and intra-run variability of <0.13 and <0.15 log10, respectively. No cross-reactivity was observed (n=51 pathogens). Regarding clinical performance, 188/189 clinical tissue samples yielded a valid result (average: 1,576,547 cells/sample, range: 145–19,428,239 cells/sample). In total, 87/189 samples tested positive (average: 673 cop/1,000 cells, range: 0.06–60,018 cop/1,000 cells), of which 14/189 were positive for multiple targets. Further evaluation is ongoing.

CONCLUSIONS

The hexaplex assay demonstrated excellent precision and linearity as well as great performance and the ability to quantify six targets simultaneously in challenging matrices, making it a promising tool to improve molecular diagnostics in tissue samples.

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020

Advancements in diagnostics

EVALUATING THE EFFICACY OF SIGMA–MM™ AT INACTIVATING MPOX VIRUS AND POTENTIAL USE IN RT-QPCR APPLICATIONS

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BACKGROUND-AIM

There is a need to render specimens safe for transportation, and for testing in circumstances with limited biological containment facilities. Sigma–MM™ has been on the market for a number of years and has been shown to effectively inactivate infectious material whist maintaining viability of Nucleic Acid (NA) from specimens infected with infected with SARS-CoV-2, influenza, RSV, enterovirus and adenovirus. In this work we are evaluating Sigma–MM™ for inactivation of Mpox virus to enable safe transportation of materials for Mpox diagnosis via a NA amplification test such as PCR.

METHODS

For viral inactivation, the Mpox viral isolate (approximate concentration 1x10e6PFU/mL) was mixed with three lots of Sigma–MM™ medium, each with four different experimental conditions as follows: 1.5mL of each buffer was added to 100ul or 500ul of virus for 1- or 5-mins incubation time, giving a total of 12 conditions. The cytotoxic component of the medium was removed using the PEG-8000 precipitation method. Survival of virus in the medium was assessed by plaque assay on 24-well plates of confluent Vero E6 cells using a 5-day incubation period with fixation using formaldehyde and staining with crystal violet.

RESULTS

All three buffers at all four conditions had no plaques present in any of the serial dilutions or undiluted resuspended pellet. Plaques were counted from the initial undiluted resuspended pellet in the controls with 100uL and 500uL of virus. We calculated an average of 5.06x10e2 and 1.32x10e3PFU/mL respectively. Therefore, the titre reduction calculated for the conditions using an initial volume of 100uL of virus was 5.06x10e2, and the titre reduction for conditions using an initial volume of 500uL of virus was 1.32x10e3.

CONCLUSIONS

Our preliminary round of testing has demonstrated that Sigma–MM™ medium is effective at inactivation of Mpox virus. This would be particularly beneficial when collecting and testing Mpox samples where there is an increased risk of exposure to the healthcare worker or in settings outside of the laboratory. The testing of compatibility with PCR is ongoing and results will be available shortly.

021

Advancements in diagnostics

EVALUATION OF ALTERNATIVE CLINICAL SAMPLES FOR DETECTION OF SARS-COV-2 RNA AND INFLUENZA VIRUS RNA

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BACKGROUND-AIM

The most commonly used and reliable test for diagnosis of SARS-CoV-2 and influenza virus is reverse transcribed polymerase chain reaction (RT-PCR). Quality of the sample, viral load and analytical sensitivity are critical for the test performance.
Aim: To compare the efficiencies of self-collected clinical samples of saliva (S) and nasal swab (ScN) with healthcare-worker-collected nasopharyngeal swab (HCW-NP) for SARS-CoV-2 + Influenza A/B RT-PCR test.

METHODS

Three clinical samples (S, ScN, HCW-NP) were collected from 404 SARS-CoV-2 and influenza virus infection suspected symptomatic cases (214 female, 190 male; median age 37), who attended Ege University and Erciyes University Hospital between November 11th 2021 – March 25 th 2022. Samples were tested with SARS-CoV-2/Influenza A/B RNA test (Cobas® SARS-CoV-2 & Influenza A/B on Cobas 6800 System; Roche Molecular Systems, USA). According to the algorithm based on identified criteria, SARS-CoV-2 or Influenza virus infection status of all patients was determined. The criteria, compatible with the prospectus, were determined by us. Positive and negative predictive values (PPV, NPV), sensitivity, specificity and coefficient of variation values (CV) of S, ScN and HCW-NP samples were analyzed. Interrater reliability of the results obtained from three different specimen types were calculated based on Cohen's Kappa values. Pearson correlation coefficient values were used to calculate the correlation of cycle threshold (Ct) results for three different positive specimen types.

RESULTS

SARS-CoV-2 and Influenza-A infection rates were 42% (n=170) and 7,9% (n=32), respectively. Especially S has a good PPV, NPV, sensitivity and specificity values, also S and ScN have almost perfect correlation (all Kappa values δ0.81; p<0.001) with HCW-NP. But Ct value correlations were weak (all values of "r": <0.55; p<0.001). All CV results showed that precision of the test was appropriate (all CV values δ6%).

CONCLUSIONS

Saliva and ScN could be alternatives to each other for qualitative results. Weak Ct value correlations didn't affect this situation. Considering the compatibility of the results, these easily collectable specimen types will provide fast, effective and early diagnosis with the correct protocol.

022

Advancements in diagnostics

FACILITATING INTER-LABORATORY COMPARISON THROUGH THE STANDARDISATION OF RESPIRATORY VIRUS MOLECULAR ASSAYS USING DDPCR.

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BACKGROUND-AIM

Viral pathogens are the most common cause of respiratory tract infection across all age groups. In severe cases, identifying the causative agent aids targeted treatment and can lead to improved clinical outcome.
Molecular methods including multiplexed assays have improved detection of viral pathogens. However, the lack of International Standards for respiratory viruses means it can be difficult to compare accuracy and reliability of molecular respiratory assays from one laboratory to another. Digital droplet PCR (ddPCR) allows for reliable quantitation of quality control materials in the absence of International standards and supports inter-laboratory comparison, method verification and validation.
This study's aim was to assess standardisation of viral load quantitation of Influenza A (INFA), Influenza B (INFB), Respiratory Syncytial Virus A (RSVA) and SARS-CoV-2 (SCV2) through the use of ddPCR within a single multiplexed format.

METHODS

Characterised clinical isolates of the four viral targets were serial diluted in suitable matrices. The series were initially assessed using individual real-time qPCR assays to establish titres and linearity. Selected titres for each viral pathogen were combined into single vial format, further characterised using real time qPCR and subsequently, characterised using ddPCR (BioRad QX200) to establish calibrated measurements in ddPCR copies/ml.

RESULTS

Initial qPCR showed variable viral load across different molecular assays depending on the type of viral target or calibrator, and/or assay used. Characterisation using ddPCR and the use of the data to normalise qPCR values resulted in harmonisation of standard curves and equivalent quantitation. This suggests that ddPCR can be used to aid standardisation of PCR workflows for viral load determination.

CONCLUSIONS

Comparison of inter-laboratory data and the validation of a respiratory molecular assay prior to its clinical application can pose challenges due to the absence of International Standards. Characterisation and testing using qPCR alone showed results dependant on aspects such as type of calibrator used. Digital PCR allows for calibration in the absence of a standard, which facilitates inter-laboratory comparison and implementation of the assay into the clinical laboratory setting.

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023

Advancements in diagnostics

FIRST STEP OF THE VALIDATION OF THE TTV-R-GENE® AS A STANDARDIZED TOOL FOR TTV DETECTION AND MONITORING IN SERUM

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BACKGROUND-AIM

The TTV R-GENE® assay (ref.423414, bioMérieux) is validated under IVDR to detect and quantify the Torque Teno Virus in whole blood and plasma. As serum samples are commonly used to study the role of TTV in various contexts, analytical performances in this specimen type were determined.

METHODS

Analytical performances of TTV R-GENE® in serum (clinical/contrived samples) were determined with EMAG® and QuantStudio™ 5/5 Dx (for LoD, linearity and precision) and verified on additional thermocyclers (multiple-amplification platforms study).
Analytical sensitivity: 20 extraction replicates of 8 TTV species (1, 6, 7, 8, 10, 24, 27 and 29 – linearized plasmids) diluted in serum to reach the claimed LoD in whole blood and plasma (250 cp/mL) were tested.
Linearity: Serial dilutions of the TTV8 linearized plasmid in serum to reach 11 concentrations with 3 extraction replicates per concentration were tested.
Precision: 2 positive panel members (at ULoQ and at LLoQ=LoD) and 1 negative panel member were tested introducing variabilities (days, operators, instruments, lots of assay) to reach 72 replicates over a 6-days period.
Multiple amplification platforms: performances equivalence between QuantStudio 5/5 Dx (as reference), LightCycler® 480 System II, Rotor-Gene Q and CFX96™/Opus 96 was assessed on 17 TTV-positive samples (13 clinical and 4 contrived samples to cover high and low loads) and 3 TTV-negative samples.

RESULTS

The LoD95% in serum was confirmed at 250 cp/mL on the 8 TTV species.
The tested concentrations for precision and linearity allowed to demonstrate a quantification range from 2.4 to 9.0 log10 cp/mL.
For the multiple-amplification platforms study, the maximum absolute difference between all tested platforms and the QS5 used as reference was 0.5 log10 cp/mL, with a maximum average of 0.2 log10 cp/mL.

CONCLUSIONS

The analytical performances of the TTV R-GENE® assay demonstrated in serum are equivalent to performances claimed for whole blood and plasma in term of limit of detection, precision and linearity. Moreover, the assay demonstrates reliable results on multiple amplification platforms: QS5, CFX96/Opus 96 , RGQ and LC480. Those results are a first step of the validation process to extend the use of the assay as a standardized tool also in serum.

024

Advancements in diagnostics

IMPORTANCE OF SEQUENCING ACTIVITY IN THE DETECTION OF NEW VARIANTS OF SARS-COV-2 IN A VENETO REGION HOSPITAL

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BACKGROUND-AIM

The fast-growing Omicron sublineage XBB 1.16 has been elevated as a new variant of interest (VOI) by the World Health Organization on April 17, 2023. It was officially named on March 5, 2023. The variant XBB 1.16 is a descendant of the recombinant XBB, that is a fusion between two BA.2 sublineages. This variant is the dominant one in India, but it was also reported in other countries. No changes in illness severity was reported with this new variant.

METHODS

All nasopharyngeal swabs for molecular detection of Sars-CoV2 were sent to microbiology laboratory of San Bortolo Hospital in Vicenza.
These samples were routinely processed by Roche® Cobas 6800 system which has two targets: ORF 1 a/b and E genes.
The swabs, that tested positive with threshold cycle (CT) less than 26, were selected random and sequenced for the surveillance of the epidemiological trend of Sars-CoV2 virus and research of a new variants.
The genome sequencing was made by Illumina® NGS platform by using primers and kits V3 model.

RESULTS

From 15/04/23 to 10/05/23 four sessions of sequencing were analyzed. Each session analyzed 24 samples.
According to results of genome sequencing it emerged that 3 samples belonged to new variant XBB 1.16. Two of these samples come from the same patient in two different sampling within five days.
The third case was a patient from the Psychyatric ward.

CONCLUSIONS

We were among the first to detect the presence of the new variant from random collection of samples and their analysis in our area. It is evident that genome sequencing activity is essential to detect new variants of SARS-CoV2 and that it should be maintained even in periods of low incidence of cases.

025

Advancements in diagnostics

ON THE DEVELOPMENT OF A MOLECULAR DIAGNOSTIC ASSAY COMBINING NUCLEIC ACID AMPLIFICATION AND A LATERAL FLOW READ-OUT: ISOTHERMAL METHODS AND POLYMERASE CHAIN REACTION AS AMPLIFICATION ALTERNATIVES

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BACKGROUND-AIM

SARS-CoV-2 has stimulated the creation of affordable, deployable, easy-to-use and accurate nucleic acid tests. Nucleic Acid Lateral Flow Assays (NALFAs) gather such characteristics, relying on the use of reporter and capture oligonucleotide probes to efficiently recognize target nucleic acid sequences by means of a colorimetric signal. NALFAs can be used to detect RNA viruses, if the sample is subject to a reverse transcription step, usually followed by nucleic acid amplification, in which the amount of target molecules is enriched. In the past, both Reverse Transcription Polymerase Chain Reaction (RT-PCR) and isothermal amplification methods like Reverse transcription Recombinase-Aided Amplification (RT-RAA) were combined with NALFAs. While RT-PCR is associated with more accuracy, isothermal amplification enables the NALFA to be deployable, more easy to perform and eventually more affordable, being interesting to explore how both methods perform with our in-house developed NALFA.

METHODS

RT-RAA and RT-PCR were independently used to produce modified amplicons of SARS-CoV-2 RdRp gene. In both strategies, the amplicons were tailed with ssDNA (single-strand DNA) sequences in the 5´-ends, to be detected simultaneously by complementary oligonucleotide probes immobilized on a NALFA strip and by oligonucleotide probes attached to AuNPs (gold nanoparticles). In order for the two methods to be established, several parameters of the NALFA were optimized, including the concentration of oligonucleotide probes, the amount of AuNPs, the type and volume of running buffer, or the concentration of amplicons. In the end, the LOD and cross-reactivity of both methods was determined.

RESULTS

NALFA parameters providing the best results included 1.5 mg/mL of oligonucleotide probe, 5µLs of AuNPs at an optical density (OD) of 5, 10µL of saline-sodium citrate (SSC) running buffers and 10µL of amplicons. The limit of detection (LOD) of the RT-RAA-NALFA assay was found to be 10-10^2 copies of RNA/µL, while RT-PCR-NALFA managed to recognize a copy number as low as 1 copy/µL, both being suitable for detection of SARS-CoV-2.

CONCLUSIONS

These results are promising for the development of two distinct, but robust diagnostic alternatives, able to mitigate future pandemics caused by RNA viruses, in the same way as SARS-CoV-2.

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Advancements in diagnostics

PERFORMANCE EVALUATION OF A CE-IVD TEST FOR DETECTION OF HEPATITIS B VIRUS IN PATIENT PLASMA

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BACKGROUND-AIM

Hepatitis B virus (HBV) causes acute viral hepatitis which can progress into a chronic infection in 5-10% of immunocompetent and in up to 20% of immunocompromised patients. Untreated chronic HBV infection can often lead to cirrhosis and increases the risk of hepatocellular carcinoma. Diagnostic tests based on real-time qPCR technology are capable of both qualitative and quantitative detection of HBV DNA in patients´ plasma and sera and can aid in diagnosis and clinical management of patients including evaluation of antiviral treatment response. Here we evaluated the clinical performance of the TaqPath™ Menu GeneProof™ Hepatitis B Virus PCR kit.

METHODS

The retrospective study was performed on 617 clinical samples which represent leftover sera from routine diagnostic testing. All samples were tested using the TaqPath Menu GeneProof Hepatitis B Virus (HBV) PCR kit. The positive sample cohort was tested in parallel with the artus® HBV RG PCR Kit (Qiagen) and the negative sample cohort with the Murex HBsAg Version 3 (DiaSorin) assay. All tests were performed according to the manufacturer´s instructions for use. Positive and Negative Percent agreement (PPA and NPA) between the TaqPath Menu GeneProof HBV PCR kit and comparators were calculated.

RESULTS

In total 503/617 samples were negative for HBV when tested with the TaqPath™ Menu GeneProof™ HBV PCR kit and corresponding results were obtained with the Murex HBsAg Version 3 assay in 100% of the samples. Of 114 samples detected as positive by the TaqPath Menu GeneProof HBV PCR kit only 1 sample showed a discordant result and tested as negative using the artus® HBV RG PCR Kit. This sample was retested and showed a weak positive result at retest using the artus® HBV RG PCR Kit, indicating that the viral concentration is likely at the limit of detection of this assay. The PPA and NPA of the TaqPath Menu GeneProof HBV PCR kit and the comparators for the detection of HBV in clinical specimens were both 100%.

CONCLUSIONS

TaqPath™ Menu GeneProof™ HBV PCR kit shows excellent clinical performance for detection of HBV infection. The test can also be used for quantitative evaluation of HBV DNA in clinical samples which can be used for infection monitoring and optimal clinical patient management.

027

Advancements in diagnostics

PERFORMANCE EVALUATION OF A NEW RANDOM ACCESS MOLECULAR TEST SYSTEM (NEUMODX 96) WITH A FULLY AUTOMATED (COBAS®AMPLIPREP/TAQMAN) CONVENTIONAL MOLECULAR TEST SYSTEM FOR HEPATITIS B VIRUS (HBV) AND C VIRUS (HCV) VIRAL LOAD ESTIMATION

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BACKGROUND-AIM

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) viral load (VL) estimation is essential in the management of both HBV & HCV infections. Due to longer turn-around –time for VL estimation, many patients drop out from the cascade of care. To achieve the global goals of substantially reducing the morbidity and mortality by HBV/HCV and moving towards their elimination by 2030, we definitely need molecular diagnostic platforms with faster, random i.e. single sample access. We evaluated the performance of a recently launched NeuMoDx (QIAGEN) random access system with the COBAS® AmpliPrep (Roche) for HBV and HCV VL estimation.

METHODS

Archived once thawed plasma samples were retrieved from –80C and tested on both the platforms. Correlations between the assays were determined by linear-regression and Bland-Altman analysis.

RESULTS

The study included samples from 186 liver disease patients. For HBV, out of 99 samples, 49 (49.49%) were DNA positive. For HCV out of 87 samples, 39 (44.82%) were RNA positive Overall in the study cohort, the Male: Female ratio was (2.5:1) with the mean age of 45 (±14.82) years.143 (76.8%) were non-cirrhotic. The median VL detected by NeuMoDX for HBV was 2.9 (IQR 2.0-4.3) log10 IU/mL and by Cobas, it was 3.7 (IQR 2.28-4.56) log10 IU/mL, with a correlation of R2=0.982. In HCV the median VL detected by NeuMoDX was 4.9 (IQR 4.2-5.4) log10 IU/mL and by Cobas was 5.1 (IQR 4.07-5.8) log10IU/mL with a correlation of R2=0.957. For both markers, 100% concordance was seen between the assays. 100 blood donor samples (HBVDNA/HCV RNA negative) were tested on both systems and 100% concordance was seen. Among HBV DNA positive, genotype (GT) information was available in 24, predominant GT was D (18, 75%) followed by GT A (4, 16.66%) and GT C (2, 8.33%). In HCV GT results were available in 38, GT 3 (25, 65.7%), followed by GT1 (6, 15.7%) and GT 4 (5, 13.1%). Both the assays demonstrated 100% concordance for HBV DNA and HCV RNA estimation across all genotypes.

CONCLUSIONS

NeumoDx assay is a good, reliable random access platform which can effectively deliver results on the same day. The most important potential application of such random access molecular assays lies in faster turn-around time of VL reports for immediate access to clinical care.

028

Advancements in diagnostics

PERFORMANCE OF ALLPLEX II HPV28 IN COMPARISON WITH ANYPLEX II HPV28 FOR DETECTION OF HIGH-RISK HUMAN PAPILLOMAVIRUS GENOTYPES.

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BACKGROUND-AIM

Human papillomavirus (HPV) testing is the key of cervical cancer screening. Recently, a new quantitative multiplex real-time PCR HPV detection kit, Allplex™ HPV28 (Seegene, Korea), has been commercialized. This study aims to evaluate its clinical performance versus Anyplex™ II HPV28 (Seegene, Korea), a semiquantitative real-time PCR kit for screening and genotyping 19 high-risk (HR) HPV.

METHODS

Between 2022 and 2023, 308 cervical samples from women with normal (n=212) and abnormal cytology (34 ASCUS; 56 LSIL; 3 ASCH and 3 HSIL) were analysed at the Department of Virology of Limoges Hospital, France. All samples were tested by both kits concurrently.

RESULTS

HR HPV DNA was detected in 78.6% of samples with Allplex, and in 77.3% of samples with Anyplex. The level of concordance between both assays was 92.2%. Positive and negative percent agreements were 95.8% and 80.0%, respectively, with an important agreement between assays (κ=0.77). Genotype-specific agreement was important for HPV58 (κ=0.76) and HPV68 (κ=0.79), and almost perfect for the 17 other genotypes (κ=0.84-0.96). No significant differences were observed in HR HPV positivity (any and specific genotypes) between both methods ($p<0.05$). Discordant results were observed in 72 cases: 47 (65.3%) were positive only with Allplex (Allplex+/Anyplex-), while 25 (34.7%) were positive only with Anyplex (Allplex-/Anyplex+). Among Allplex+/Anyplex- cases, HPV53 (10/47) and HPV68 (9/47) were the most frequent, and 97.9% (46/47) exhibited low viral loads (mean Ct-value per genotype was >35). Among Allplex-/Anyplex+ cases, HPV39 (5/25) and HPV66 (4/25) were the most frequent, and 96.0% (24/25) were detected at the lowest signal intensity (+). Allplex viral load quantification was well correlated with Anyplex viral load semi-quantification, with Ct values of 35-40, 28-35, and 17-28 corresponding to +, ++, +++ signal intensity scores, respectively.

CONCLUSIONS

Overall performance of Allplex is similar to Anyplex, with a slightly higher detection rate of HR HPV. In samples with low viral loads, Allplex seems to be more sensitive in detecting HPV53 and HPV68, whereas HPV39 and HPV66 were more frequently detected with Anyplex.

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029

Advancements in diagnostics

THE IMPACT OF NASOPHARYNGEAL MICROBIOME IN COVID-19 INFECTION SEVERITY

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BACKGROUND-AIM

Background: The study of the human microbiome is an important tool for understanding the course of different diseases and their clinical outcome. The aim of the present study is to analyze the human nasopharyngeal microbiome in order to detect its specific features that could potentially be useful as biomarkers of the severity of COVID-19 infection.

METHODS

Methods: 43 nasopharyngeal swabs were obtained from patients with laboratory-confirmed COVID-19 infection, with a mean age 58.2 years (11-93 years), while 65.1% of them were male. Sixteen of the patients had mild symptoms (cough, fever, myalgia, weakness), while 27 patients were hospitalized, out of which 2 in Intensive Care Unit.
Extraction of the genetic material was performed using the MagMAX™ Viral/Pathogen Kit (Thermo Fischer Scientific) according to the manufacturer's instructions. RT-PCR was performed using the TaqPath™ 1-Step RT-qPCR Master Mix diagnostic kit (Applied Biosystems), according to the manufacturer's instructions. New generation sequencing was performed by amplifying the 16S rRNA gene using the analyzer (Ion PGM System, Thermo Fischer Scientific). Statistical analysis was performed using the statistical programs EZBioCloud, Rhea, LEfSe, and the PERMANOVA test.

RESULTS

. Results: According to the results, 7 bacterial Phyla were detected: Firmicutes (41.2%), Proteobacteria (28.4%), Actinobacteria (21.5%), Bacteroidetes (4. (4.7%), Saccharibacteria TM7 (2.8%), Fusobacteria (1.2%), and Spirochaetes (0.2%). Moreover, 61 bacterial families, 132 genera and 389 species were detected, with Streptococcus oralis (10.52%) being the most abundant one. The genera detected most frequently in patients with mild symptoms were Staphylococcys, Streptococcus, Prevotella and Dolosigranulum, while the microbial genera detected most frequently in hospitalized patients were Corynobacterium, Actinomyces, Anoxybacillus, and the species Streptococcus peroris, and Staphylococcus aureus. Regarding ‹-diversity, no statistically significant difference was found between patients with mild and severe symptoms, in contrast to ®-diversity, which showed statistical significance.

CONCLUSIONS

Conclusion: Although the literature in this area is limited, this study will form the basis for further research in this field, both in Greece and globally.

030

Advancements in diagnostics

TORQUE TENO VIRUS AS A BIOMARKER IN RENAL TRANSPLANT PATIENTS

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BACKGROUND-AIM

In renal transplant recipients, balancing the risk and potential benefits of reducing immunosuppression (IS) is challenging. Generally, antiproliferative agents are first reduced followed by a reduction in calcineurin inhibitors, Torque Teno virus (TTV), a non-pathogenic ubiquitous DNA virus is increasingly used as a marker of IS status. We have previously analysed the relationship between BK virus and TTV titres after IS reduction. The current work describes the implementation of TTV assay in our laboratory as well as further preliminary virological results in our patient cohort.

METHODS

20 renal transplant patients with stored blood samples were included in the study cohort from a population transplanted between 2017-19 on standard IS treatment. TTV DNA was extracted from stored frozen plasma and PCR was performed with Thermofisher 7500 Fast platform & TTV R-GENE® kit. Data was collected from electronic patient records & analysed with R 4.0.3.

RESULTS

TTV titre is a sensitive predictor of BK viral load when compared with tacrolimus plasma concentration. . 5 patients who had lost their graft in the mean of 2.8 years follow up had significantly low TTV titres ($p < 0.05$) at baseline suggesting suboptimal IS burden. In this small cohort, there was no relationship between TTV titres and rejections. Technically, the TTV R-GENE® kit was implemented successfully and could be used as part of our routine work if the clinical utility of TTV is demonstrated. An ongoing adequately powered prospective study is ongoing and will be able to address the clinical utility of TTV as a biomarker more effectively.

CONCLUSIONS

TTV titres can be reliably quantified using the TTV R-GENE® kit and they can accurately indicate the effect of IS changes on BK titre as a surrogate of its potential use as a biomarker to guide IS modification instead of the current empirical management post renal transplantation.

031

Advancements in diagnostics

VIROLOGICAL CHARACTERIZATION OF HIV-1 RNA ELEMENTS IDENTIFIED IN A SUBSET OF PATIENTS DETECTED EXCLUSIVELY THROUGH THE LTR REGION BY THE DUAL-TARGET APTIMA HIV-1 QUANT DX ASSAY

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BACKGROUND-AIM

In a little subset of HIV-patients resulting with suppressed viremia based on the pol region amplification, Aptima HIV-1 Quant Dx Assay (Aptima), a dual-target (pol and LTR) test for monitoring HIV-RNA, detects HIV-1 viral loads (VL) exclusively through amplification of the LTR-target. Virological characteristics of LTR-detected HIV-RNA elements were analyzed in deep to better understand the meaning and implications of this singular viremia.

METHODS

LTR-detected elements were isolated from plasma and peripheral blood mononuclear cells (PBMC) and evaluated for ability to infect PBMC from healthy donors. The morphology of viral pellets and the ultrastructural characteristics of PBMC obtained from samples with LTR-detected HIV-RNA were examined by electron microscopy. LTR-elements, extracted from plasma specimens, underwent Sanger sequencing. Exosomes content was also examined with Aptima.

RESULTS

In-vitro, the HIV-RNA detected only through the LTR amplification cannot trigger new cycles of infection, neither cytopathic effects nor syncytia, even at high VL (e.g. >10,000 copies/mL). At electron microscopy, plasma pellets and PBMC from patients with LTR-detected VL showed atypical vesicles. Sanger sequencing of LTR-detected HIV-RNA elements, found free in plasma or associated to cell debris (not in exosomes), provided no results.

CONCLUSIONS

In some HIV-infected patients, Aptima recognizes VL detected only through the LTR amplification otherwise not recognized with the dual-target assays based on single channel reading. We demonstrated that the HIV-RNA elements detected through LTR represent small partial/incomplete HIV-RNA transcripts not able to trigger productive infection or to transmit HIV infection. The discrimination of viremia based on LTR-only signal is essential to avoid inappropriate decisions, such as repeating of VL measurement and switch of ART, as suggested by international guidelines for monitoring of HIV-infected patients in case of viral rebound. Clinicians and manufactures of dual-target tests should consider this important feature for relevant implications in clinical management of HIV infected patients.

032

Clinical cases

A LONGITUDINAL STUDY OF SARS-COV-2 VARIANTS WITH ASSOCIATION BETWEEN VIRAL LOAD AND SYMPTOMS

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BACKGROUND-AIM

Since late 2019, US Centers for Disease Control and Prevention (CDC) reported more than 760 million confirmed cases of Sever Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) so far.
The scientific community had previously reported both presence and absence of correlation between viral load and disease severity in COVID-19 patients.
The purpose of this study was to further investigate the progression of viral load detected in real-time PCR and severity of symptoms across different SARS-CoV-2 variants in a longitudinal study cohort, tested from the onset of the disease until the demonstration of negative results to a molecular test.

METHODS

The patient cohort of this study involved 44 individuals. Patients were enrolled between December 2021 and the beginning of 2023 in Lombardy and Piedmont regions (Italy, EU) after confirmation of SARS-CoV-2 virus infection.
Each patient donated a self-collected nasal swab in 3 mL UTM vial each day and recorded the symptoms on a daily basis.
Viral load (as measured by Ct value) was determined with Simplexa™ COVID-19 & Flu A/B Direct Kit. For SARS-CoV-2 variant discrimination, we used Simplexa® SARS-CoV-2 Variants Direct kit and confirmed using a sequencing assay.

RESULTS

The cohort enrolment, considering the entire duration of the study, allowed monitoring of different SARS-CoV-2 variants, such as Delta, Omicron BA.1, BA.2, BA.4, BA.5 and the most recent outbreak of Omicron XBB.1.5.
Interestingly, our results showed no direct correlation between PCR cycle threshold (Ct) and degree of symptoms observed. Severe symptoms were in some cases associated with high viral load (Ct<20), but in several other cases, a high viral load corresponding to an absence of any visible symptoms was recorded.

CONCLUSIONS

Determining whether high viral load is a trustable asset to evaluate the disease severity of patients is of extreme relevance, since it may be crucial in the management of intensive care departments in hospitals. Our results suggested that the viral load may not be directly associated to disease severity, for any of the SARS-CoV-2 variants tested. Moreover, the study showed that asymptomatic patients can have sustained high viral load with important implications for hospitals and specific community settings.

033

Clinical cases

A PERPLEXING CASE OF ENCEPHALITIS IN AN IMMUNOCOMPROMISED TRANSPLANT PATIENT

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BACKGROUND-AIM

We describe an immunocompromised transplant patient who developed encephalopathy of unknown aetiology.

METHODS

Magnetic resonance imaging (MRI) and electroencephalogram (EEG) findings complicated the clinical picture with the patient requiring testing for variant Creutzfeldt–Jakob disease (vCJD).

RESULTS

As this was negative, an extended viral cerebrospinal fluid (CSF) panel was performed which demonstrated Human Herpes Virus-6 (HHV-6) Encephalitis. This was further complicated by deciphering whether this is chromosomally integrated HHV-6 or a primary HHV-6 Encephalitis. During a prolonged admission she represented a diagnostic challenge and management dilemma involving a variety of medical specialties.

CONCLUSIONS

We aim to explore the difficulties surrounding the diagnosis and management of HHV-6 Encephalitis in view of the masking by chromosomally integrated HHV6 and side-effects of severe cytopenias via HHV-6 antiviral treatment. We aim to elaborate on how this rare case provided new insight into the management of HHV-6 Encephalitis in severely immunocompromised transplant patients and the need for close multi-disciplinary collaboration.

034

Clinical cases

CO-INFECTION BY TWO DIFFERENT SARS-COV2 VARIANTS: A CASE REPORT

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BACKGROUND-AIM

A patient make an access to emergency room of San Bortolo Hospital in Vicenza, for Sars-CoV2 infection suspected in 15/03/23. The patient underwent a nasopharingeal swab analyzed by a molecular Point of Care Test (POCT). This test resulted positive. The next day, she was trasferred into geriatric department, in order to monitoring of Sars-Cov2 infection.
In addition, a series of molecular swabs were sent to microbiology department of San Bortolo hospital in the following days 21/03/23, 27/03/23 e 21/04/23 in order to monitoring the viral load of infection.
The results of each swabs testing were positive.

METHODS

The swab tested at the emergency room in 15/03/2023 was analyzed with ID Now Abbott method, which is a molecular rapid assay that gave results in only 13 minutes and one minute in addition for manual procedure. While samples tested in days 21-03/23, 27/03/23 and 21/04/23 were processed in microbiology laboratory with CEPHEID® XPERT®XPRESS instrument that research E and N genes. The viral loads of these 3 samples were characterized by thereshold cycles (CT) less than 26, such as to allow the genome sequencing of Sars-CoV2 virus.
The genome sequencing was made by Illumina® NGS platform by using primers and kits V3 model.

RESULTS

From the analysis of genomic sequencing these samples showed:
- 21/03/23 exhibited CH.1 variant
- 27/03/23 exhibited CH.1 variant
- 21/04/23 exhibited XXB.1.9.1 variant

CONCLUSIONS

According to results of genomic sequencing it was emerged that the patient was affected by the same Sars-CoV2 variant both 21/03/23 test and 27/03/23 test.
During the hospitalization in geriatric department the patient had another reinfection with XXB.1.9.1 variant of Sars-CoV2. This variant belonged to cluster emerged in geriatric department few days before hospitalization of this patient.
The patient didn't result negative to Sars-CoV2 until 24/04/23.
It can therefore be concluded that the patient has been through a double infection of Sars-Cov2 virus belonging to two different variants.

035

Clinical cases

HEPATITIS B MUTANT IN A DIALYSIS PATIENT.

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BACKGROUND-AIM

Hepatitis B mutant was detected in a dialysis patient

METHODS

Patient on haemodialysis with past hepatitis B was screened negative for 4 months with HepB SAg. Following a HBV PCR, HBV detected.

RESULTS

Sample sent to reference lab for sequencing and mutant detected

CONCLUSIONS

Lookback performed. However existing infection control guidance for dialysis units refers to sAg positive patients or OBI patients, rather than patients with sAg mutants

036

Clinical cases

PULMONARY KAPOSI-HIV-RELATED: A CASE REPORT

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BACKGROUND-AIM

Kaposi Sarcoma (KS) is an AIDS-related tumor. HHV8 is required to develop KS, but only a minority of infected people will develop it. KS presents with mucocutaneous disease, but may advance to extensive visceral disease. Early presentation may manifest with respiratory signs and symptoms.

METHODS

To detect HHV8 viremia we used ELITe MGB® HHV8 kit: a real time PCR. The test is validated for whole blood (WB) and plasma in EDTA. No data are available with DNA extracted from biopsies or other samples.

RESULTS

We describe a KS in a newly diagnosed HIV patient. The patient presented to the Emergency ward with fever, cough, thoracic pain, haemoptysis. RX showed lung thickening, the BAL was negative for Mycobacterium spp but yielded Pseudomonas aeruginosa. He was diagnosed with pneumonia and treated with antibiotics. His serum was reactive to HIV-1, he got hospitalized at the Infectious Diseases ward.
Further exams presented leukopenia, CD4/CD8 ratio 0, HIV-1 viremia 1650000 copies/ml. The respiratory sample (BAL) was tested for Pneumocystis jirovecii: negative. In the following days HHV8 viremia in WB detected 5068 copies/ml. We decided to re-test the BAL even though our HHV8 Kit is not validated for it, yet we detected the virus.
Computerized tomography (CT) showed disseminated nodules in the lungs: pulmonary and bronchial biopsies resulted compatible for KS.
The patient started antiretroviral therapy (ART). HIV viremia reduced and leukocytes normalized. The patient got discharged from the hospital and started chemotherapy with doxorubicin, his last HHV8 viremia is 7908 copies/ml.

CONCLUSIONS

HHV8 viremia detection has been useful to suspect KS. We have been able to find the virus in the BAL even though the method is not validated, in such samples it seems more useful a qualitative analysis than a quantitative one. The possibility to test the biopsies for HHV-8 could have been also clinically significant.
Clinical relevance of HHV8 viremia monitoring is still controversial, our data are not decisive, since our patient is still in follow-up.

037

Clinical cases

A RETROSPECTIVE REVIEW OF PARVOVIRUS B19 VIRAEMIA IN RENAL TRANSPLANT PATIENTS IN A LONDON TRANSPLANT CENTRE

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BACKGROUND-AIM

Parvovirus B19 infection, a self-limiting illness in immunocompetent hosts, can establish chronic infection in renal transplant recipients and cause significant morbidity in this immunosuppressed population. We present a study of all renal transplant recipients diagnosed with Parvovirus B19 infection at our tertiary centre over a 10-year period (n=10).

METHODS

We identified all incidences of Parvovirus B19 viraemia in renal transplant recipients over a 10-year period, confirmed by PCR in blood. Only patients with 2 or more significant positive PCR results were included in the analysis. Data on transplant indication, past medical history, and type of transplant were extracted from electronic patient records. We analysed the effect of changes in dosage and prescription of immunosuppressive drugs, number and dosage of intravenous immunoglobulin (IVIG) infusions and number of blood transfusions on haemoglobin levels, reticulocyte count and serum levels of Parvovirus B19 DNA from the time of transplant to the last available follow-up.

RESULTS

Time from transplant to first detectable Parvovirus B19 DNA ranged from 7 weeks to 16 years, with a median of 10 weeks. Of the 10 renal transplant recipients we studied, 9 received at least 1 course of IVIG with 5 receiving multiple courses (with a maximum of 12 courses) whilst 4 were treated with IVIG only once. IVIG was often effective in reducing Parvovirus B19 DNA levels and increasing reticulocyte count, especially in patients with particularly high viral load prior to treatment. However, in no patients who received multiple courses of treatment was IVIG effective in all instances. All patients maintained detectable levels of Parvovirus B19 DNA in blood throughout the entirety of the follow-up period. We found no clear relationship between the type or dosage of immunosuppressive drugs and Parvovirus B19 DNA levels in blood.

CONCLUSIONS

The heterogeneous antibody profile offered by different batches of IVIG may explain why IVIG does not reliably bring about a reduction in Parvovirus B19 DNA levels. Further work is needed to improve understanding of how immunosuppressive medications impact the susceptibility to, and severity of, Parvovirus B19 infection following renal transplantation.

038

Emerging and re-emerging viruses

MPXV GENOME MICROEVOLUTION WITHIN MPOX CASES

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BACKGROUND-AIM

In 2022, an unprecedented outbreak of monkeypox virus (MPXV) infection, MPXV-2022 outbreak has piqued the attention towards the virus cross-species spread. In the last three years, the quick diffusion worldwide highlighted that viruses spread is a silent and constant process in which the evolutionary dynamics has had a significant role. Recently, the onset of minor intra-lesion single nucleotide variants (SNVs) have been described along the transmission chain, suggesting an ancestral intra-patient minor variation. Till now, it is not clear if the contemporary presence of different SNVs with different frequencies in the same lesion could be due expecially to APOBEC3-driven evolution or to a possible co-infection.

METHODS

Here, we report the phylogenetic and mutation analysis through next generation sequencing of four epidemiologically unrelated mpox cases detected in Verona (Italy). Three of them were imported cases and one had no travel history.

RESULTS

Our analyses highlighted the presence of the current circulating strain Clade IIb, sublineage B.1 in all the patients, consistently with other reports. A total of 37 mutations, both novel and known, recorded in different countries were detected. Among them we identified three novel nonsynonymous SNVs in OPG056 and OPG105 genes, known to be prone to mutations. For three patients, we found different viral sequences in the same sample. In particular, we identified five different SNVs in co-presence with wild type allele.

CONCLUSIONS

Among the observed mutations, several SNVs are located in genes involved in immune evasion mechanisms and/or viral fitness that are consistent with a possible APOBEC3-mediated sequence editing. Further investigations on larger clinical cohorts are needed to confirm our observations and to monitor the possible spread of these new mutations. Moreover in vitro culture experiments should be performed to evaluate if the observed mutations can favour virus replication.

039

Emerging and re-emerging viruses

CIRCULATION OF WEST NILE VIRUS IN ALGERIA: SEROPREVALENCE, MOLECULAR EPIDEMIOLOGY AND NEUROINVASIVE-RELATED DISEASE

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BACKGROUND-AIM

West Nile Virus (WNV) is an arbovirus transmitted by mosquitoes of the genus Culex.Human infection presents often with a febrile illness, but can express neuro-invasive disease of varying severity. WNV is widely spread in the Mediterranean basin. In Algeria, the available data on WNV are limited.The objective of this work was to study the circulation of WNV in different geographical regions of the country.

METHODS

Five-year study was conducted from 2017 to 2019 in five regions of Algeria.The first part of the study was to determine the seroprevalence of WNV infection and to identify associated risk factors. Serological analysis was performed by ELISA for anti-WNV IgG , followed by seroneutralization. Afterward, the study aimed to determine the frequency of WNV neuroinvasive disease (WNVND) during the period of vector activity. Virological diagnosis was done following ECDC laboratory criteria. Lastly, molecular characterization was done on WNV strains obtained from patient samples and mosquitoes.

RESULTS

A total of 1235 serum samples was collected and tested. The overall seroprevalence of WNV infection was estimated to be 21.9% by ELISA and 16.9% by PRNT. Multivariate analysis identified residence in the South and living in a rural/suburban setting as risk factors for infection independently of the other studied variables. 31 WNVND cases were confirmed in the laboratory, particularly in patients over 10 years of age (30/31 cases), with a predominance of aseptic meningitis (74.1%). Most cases were recorded between September and October, mainly in the north of the country , either in rural or suburban areas. WNV lineage 1 was identified in three studied regions, and lineage 2 in only one. Phylogenetic analysis revealed that the four lineage 1 sequences obtained in this study belong to clade 1a and to the Western European and Mediterranean geographical subgroup.

CONCLUSIONS

The results obtained in this study allow to conclude that the circulation of WNV is endemo-epidemic in Algeria, particularly in the south of the country, with a higher frequency of WNVND in the northern regions. It is essential to continue monitoring this arboviral disease in Algeria and to include it in the differential diagnosis of neuroinvasive viral infections during the summer-autumn season, particularly in adults.

040

Emerging and re-emerging viruses

COMPARISON OF COMMERCIAL PLATFORM FOR MONKEYPOX VIRUS INFECTION DIAGNOSIS

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BACKGROUND-AIM

Since May 2022, more than 87,000 global cases were confirmed. Monkeypox virus (MPXV) infection must be confirmed by polymerase chain reaction (PCR) assay.
This study aims to compare RealStar® Orthopoxvirus PCR Kit 1.0 (altona DIAGNOTICS, Germany – RS) and STANDARD M10 MPX/OPX (SD BIOSENSOR, South Korea – M10) RealTime-PCR assays for MPXV laboratory diagnosis.

METHODS

Frozen samples from 205 subjects were selected and stratified according to routine test results by RS (RS-1): in details, 100 was negative skin lesions (SL) and 200 were positive samples at variable stage of infection (100 SL, 42 plasma-PL, 52 other biological matrixes-OBM). Positive samples were retested with RS (RS-2). RS is a RT-PCR targeting A3L gene; M10 is a single test cartridge, targeting E9L (OPX specific), G2R (MPXV specific) and two overlapping regions (Clade specific): the result is invalid with no Internal Control amplification or negative E9L. Invalid tests were repeated. Positive and Negative Percent Agreement (PPA, NPA) were calculated. Clade was attributed by Monkeypox Virus Real Time PCR Kit (Jiangsu Bioperfectus Technologies Co., Ltd, China), with 100% Clade-II.

RESULTS

The median (IQR) Ct values of RS and M10 (OPXV target) assays were highly similar in positive samples. The PPA of M10 compared to RS-1 was 89.5% considering system interpretation, and 96.0%, when the operator classified results as positive if any target was detected; NPA was 100%. Comparing RS-2 run and M10, an overall concordance of 95.3% between assays was found; however, considering operator interpretation, M10 returned more positive results than RS-2. The loss in positive occurrences for both RS-2 and M10 was associated to low viral concentration samples, with a possible influence of thawing. All samples collected at the time of Mpox diagnosis were positive, as expected, with both RS-2 and M10 and M10 correctly attributed the Clade (West-Africa/II).

CONCLUSIONS

M10 MPX/OPX assay demonstrated high reliability and analytical sensitivity for detection of MPXV DNA and Clade attribution.

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Emerging and re-emerging viruses

DATA FROM QCMD POXVIRUSES EXTERNAL QUALITY ASSESSMENT (EQA) PILOT STUDY, 2022/2023

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BACKGROUND-AIM

Mpox (monkeypox, MPX) is a viral zoonosis with symptoms like smallpox, but clinically milder. It primarily appears in tropical rainforest areas of central and west Africa, however multiple outbreaks occurred globally in 2022, unrelated to travel or animal trafficking. The disease is caused by MPXV, a double-stranded DNA virus of the Orthopoxvirus genus (Poxviridae), classified into two clades (clade I and II, with subclade IIb incl. current outbreak variants).
Due to the 2022-23 global mpox outbreak and increasing demand for laboratory preparedness (especially in non-endemic countries), in autumn 2022 QCMD first offered a pilot EQA scheme to support laboratories. The aim was to evaluate the current use of molecular technologies for the detection of MPXV within the clinical laboratory setting. While QCMD will continue to provide this pilot study in 2023, here we review the results of the first three challenges conducted in 2022/2023.

METHODS

The panels contained different viral concentrations and strains from both clade I and subclade IIb, along with specificity samples of closely related orthopoxviruses (vaccina, VACV and cowpox virus, CPXV), to allow assessment of sensitivity and specificity of molecular assays. The panels were distributed to 254 laboratories in 36 different countries, in total for the current challenges. The results were collected through QCMD's dedicated ITEMS online reporting system, before being analyzed to determine laboratory performance.

RESULTS

Demand for this new scheme was driven by clinical laboratories need to rapidly introduce an assay to manage patients with suspected mpox. Laboratories used both in-house assays (32.2 %) and commercial assays (67.8 %) for testing. Qualitative assessment of the reported results showed that commercial and in-house assays performed similarly. Several commercial assays were pan-orthopox and unable to differentiate MPXV from related orthopoxviruses.

CONCLUSIONS

As MPXV still constitutes a public health emergency of international concern it is important that laboratories can accurately detect and differentiate between closely related pathogens, particularly in high-risk patients and close contacts to prevent spread and manage patients accordingly.

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Emerging and re-emerging viruses

DESIGNATION AND EVALUATION OF CELL-BASED ANTI-VIRAL SCREENING SYSTEM WITH PSEDOTYPED VIRION OF VIRAL HEMORRHAGIC FEVERS

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BACKGROUND-AIM

Since 2015 to date, Viral hemorrhagic fever (VHFs) are in the list of top priority diseases for epidemic preparedness announced by WHO. Due to continuous outbreaks in Africa countries with high infectivity and mortality rates over 20~50%, it would concerned as potential risk on global public health, to Korea as well. Moreover there is no reliable and commercially available vaccine or therapeutics for VHFs.

METHODS

In this study, we developed a cell-based screening system for identifying and evaluation of anti-viral agents to Lassa fever viruses (LAVs). As considered the genomic variance of LAVs between subtypes, we selected 9 strains isolated from human infection from lineages I~VII and optimized the condition of pseudotyped LAV-GP expressing virions.

RESULTS

Compared to control (VSV-G), the infectivity of LV-GP pseudotyped virions determined as higher, at least 10^6 RLU. Next we designed cell-based screening condition with pseudotyped LV-GP virions and evaluated using active compounds reported as anti-viral compounds to LAVs. It showed the virions-related activity was decreased only in compound–treated cells which has the inhibitory action to virus entry, whereas no change in replication-inhibitory compound. The evaluated results of individual active compound presented different inhibiting action depends on lineages. And then, we optimized the screening platform in quantity by mean of luminescence activity to identify anti-viral compounds inhibiting LAV-GP pseudovirus infection into cell. From the applied with compound library to this screening system, several candidates seems to be inhibited Lassa virus infection, but it needs to confirm its anti-viral action in characters.

CONCLUSIONS

These results suggested that developed cell-based screening platform is capable for an identification of anti-viral agents as general-purpose or lineage-specific to LVs. In addition, this platform could be applied to develop an evaluation system for therapeutics candidates against emerging pathogens in Korea.
This research was supported by the “National Institute of Health” research project (No. 2020-NI-013-02 and 2023-NI-013-00).

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Emerging and re-emerging viruses

DETECTION OF WEST NILE VIRUS AND INSECT-SPECIFIC VIRUSES IN CULEX MOSQUITOES IN CENTRAL MACEDONIA, GREECE, 2020-2022

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BACKGROUND-AIM

Since 2010 West Nile virus (WNV) is endemic in Greece, with Central Macedonia (CM) being one of the most affected Regions. Aim of the present study was to test for WNV Culex spp. mosquitoes collected in CM during 2020-2022, initially to estimate the infection rate, identify the potential endemic foci, and genetically characterize the circulating strain(s) of the virus, but also to analyze the virome of mosquitoes.

METHODS

During May to October 2020-22, 57,709 Culex spp. Mosquitoes were collected from 87 sites in the seven regional units of CM. Mosquitoes were grouped into 1443 pools based on the site collection and date of collection. For WNV detection a commercial real time RT-PCR was applied. An amplicon-based next generation sequencing (NGS) protocol was applied on the WNV positive samples with Ct value<28, while de novo NGS was applied on representative pools of each year. Phylogenetic analysis was done using MEGA 11.

RESULTS

WNV was detected in 91/1443 (6.31%) mosquito pools. During 2022 urban areas were highly affected. All WNV sequences clustered into the Central European clade of WNV lineage 2. Whole genome sequences were taken from 33 samples. The strains of 2020-2022 were evolutionary variants of a strain introduced in the country in 2019. From the metagenomic analysis viruses belonging to six families and few unclassified families were identified.

CONCLUSIONS

An intense circulation of WNV was observed in CM Region in Greece during 2019-2022 with differences in geographic distribution and monthly infection rates in the regional units. The virome analysis identified a plethora of viruses, some of them detected for the first time in Europe.
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Emerging and re-emerging viruses

DEVELOPMENT OF A PLAQUE REDUCTION NEUTRALIZATION TEST FOR SARS-COV-2 USING PSEUDOTYPE VIRUS

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BACKGROUND-AIM

The aim of out projeci to develop a standardized pseudovirus PRNT test format according to classical PRNT by creating pseudoviruses expressing spike proteins of SARS-CoV-2 variants.

METHODS

In this study, SARS-CoV-2 spike pseudotype virus was produced using vesicular stomatitis virus vector. Vaccinated human plasma samples (n=13) and healthy negative controls (n=2), previously studied PRNT, with or without COVID-19, pseudoviruses of three different SARS-CoV-2 variants generated in the study (Wuhan, Alpha and Beta were tested under BGD-2 conditions using omicron).

RESULTS

Plasma neutralizing antibody titer as measured by the Wuhan lineage pseudovirus test, using live SARS-CoV-2. It correlated well with values measured by PRNT (R2 = 0.7). The pseudovirus neutralization antibody titers between variants differed as expected.

CONCLUSIONS

As a result of this study, a VSV-based pseudoneutralization test format that can be performed under BSL-2 conditions was developed, optimized and made ready for use.

045

Emerging and re-emerging viruses

EMERGENCE OF A NEWLY DISCOVERED TICK-RHABDOVIRUS IN CANADA

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BACKGROUND-AIM

Climate change influences on the global distribution of tick species vectoring pathogens. Screening field-collected ticks for arboviruses is a powerful method of detecting tick-borne viruses (TBVs). This will provide 'early warning' detection for pathogens that might be introduced by invasive species or migratory birds carrying ticks.

METHODS

Ticks were collected at 15 different sampling sites in 3 western provinces in Canada (May to October, 2022). A total of 168 ticks were collected by dragging method or directly from hosts. Genes were extracted from homogenized ticks. Tick samples were identified by morphological keys or molecular techniques. A Pan RT-PCR protocol was used to screen ticks for Rhabdoviridae family. PCR products were visualized by QIAxcel, then positive samples were sent for Sanger sequencing. Evolutionary relationships of sequences were determined by phylogenetic tree using Geneious.

RESULTS

Of 168 ticks, 87 were collected by dragging method and 18 from mammalians, 28 from humans, and 35 from migratory birds. Tick species were identified as De. albipictus, De. andersoni, De. variabilis, Ix. auritulus, Ix. banksi, Ix. cookei, Ix. pacificus, and Ix. spinipalpis. Five tick specimens (all De. andersoni) were positive for Rhabdoviruses. The genetic characterization of the 5 Rhabdovirus sequences showed highest similarity (71%) to a newly discovered Rhabdovirus from the USA named "American dog tick rhabdovirus-2" isolated from De. variabilis in 2016.

CONCLUSIONS

We report the first detection of a novel tick-Rhabdovirus in Canada. We postulate that the name of "American dog tick Rhabdovirus" was initially selected for this novel Rhabdovirus in the USA due to the common name of De. variabilis, which is American dog tick. However, our finding of the detection of this novel Rhabdovirus in De. andersoni in Canada suggests that this virus is not restricted to De. variabilis, as it was initially supposed, and perhaps a more generic name for this Rhabdovirus, tentatively tick-Rahbdovirus, would be more accurate. These findings improve the current knowledge of the genetic diversity, classification and evolution of Rhabdovirus family. More research is needed to determine the potential significance of this novel tick-Rhabdovirus as a pathogen for human or veterinary health.

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Emerging and re-emerging viruses

ENGINEERING OF CELL-BASED EVALUATION SYSTEM FOR ANTI-VIRAL AGENTS TO RIFT VALLEY FEVER VIRUS (RVFV)

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BACKGROUND-AIM

Viral hemorrhagic fevers (VHFs) including Ebola are severe viral hemorrhagic illness in human and other primates. VHFs regards as one of priority diseases for epidemic preparedness listed by WHO since 2015. Among VHFs, Rift valley fever (RVF) is a zoonotic arthropod-borne emerging infectious diseases endemic to sub-Saharan Africa. Given considering the emergence situation of MERS and COVID-19 outbreak in Korea, it is concerned as potential risk of threat to public health in Korea.

METHODS

Since there are no effective vaccine or specific therapeutics for RVFV infection in human and its high-risk of biohazard level as of BSL-3, we aimed to develop cell-based screening system for anti-viral agents against RVFV, which is applicable to BLS-2 facility. We designed two types of recombinant virion system, pseudotyped and T7-RNA polymerase driven minigenome assay (MG). To engineer both system, the target antigen; glycoprotein (GP), nucleocapsid protein(NP) and RNA dependent RNA polymerase (RdRp) of RVFV was codon-optimized and constructed into proper expression vector in each system.

RESULTS

The recombinant-vector expression was optimized the conditions for enhance the production of active pseudotyped RVFV-GP virions. In part of minigenome-based assay, we were established the system based on viral genomic S and M segment of RVFV, respectively and assessed the relative activity by Nano-Luc expression compare to control without applied any of target genes of RVFV.

CONCLUSIONS

In here, we have established the functional recombinant virion-based system that will be used for cell-based anti-viral screening platform in RVFV infection; in particular stages of virus entry and replication.
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Emerging and re-emerging viruses

EVALUATION OF RAPID SYNDROMIC MOLECULAR PANEL IN THE DIAGNOSIS OF PATIENTS WITH CENTRAL NERVOUS SYSTEM INFECTION

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BACKGROUND-AIM

The aim of this study is to evaluate the FA-ME panel in the early diagnosis of patients admitted with central nervous system (CNS) infection as well as to determine the prevalence of microbial agents detected by the panel.

METHODS

In this cross-sectional study, an analysis of records of 1588 CSF samples sent to the microbiology laboratory at Ege University Hospital between 01.06.2018- 31.08.2022 was conducted. All samples were tested using the FilmArray panel (BIOFIRE, Salt Lake City, UT) which can detect 14 pathogens (7 viral, 6 bacterial and 1 fungal). To confirm all positive results, conventional methods were used and the medical history of patients who had been monitored for CNS infections was reviewed. Age, gender, and the clinical service that provided samples were considered as independent variables in this study. For the analysis of positive cases and independent variables, the Chi-square test was used. The significance level is accepted as $p<0.05$.

RESULTS

73% of the patients were adults. Males constituted 59.4% of children, whereas females accounted for 51.2% of adults.
Of the total 1588 samples, 127 (7.9%) were positive. In comparison with confirmation methods, 10 (7,8%) of the panel results as false positives and 17 (13,4%) were of the panel results were considered as bystanders. After excluding these results, the true positivity rate was found to be 6.3% (100/1588). According to the corrected distribution of causative microorganisms, viruses accounted for 58%, bacteria 39%, and fungi 3%. The most common viral agent was HSV-1, while the most common bacterial agent was S. pneumoniae. The positivity rate was significantly higher in males (7,8%) (p=0.008).
It was found that one CSF sample was negative on the panel, but positive by antigen test for C neoformans/gattii.The test's sensitivity and specificity were 99.2% (95% confidence interval, 96.3%- 100.0%), and 99.3%, (95% CI, 98.8%- 99.7%) respectively. The positive predictive value was 92.1% (95% CI, 86.6%- 96.0%) while the negative predictive value was 99.9% (95% CI, 99.7%- 100.0%).

CONCLUSIONS

The FilmArray Panel has the advantage of diagnosing CNS infections in a very short period of time, but it is essential to evaluate the patient's clinical condition and CSF biochemistry results at the same time in order to prevent false positives.

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Emerging and re-emerging viruses

EVALUATION OF THE PERFORMANCE OF THE ALINITY M MPXV ASSAY

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BACKGROUND-AIM

Timely testing of monkeypox virus (MPXV) is important for patient care, contact tracing, and decreasing transmission. The new Alinity m MPXV Research Use Only (RUO) assay developed for direct qualitative detection of MPXV DNA in clinical specimens was evaluated by comparison with a MPXV in-house assay established with start of the global outbreak in summer 2022.

METHODS

Residual archived swab samples tested with the in-house assay in 2022 were selected based on previous test results (100 negatives, 300 positives) and were diluted 1:10 to obtain sufficient material for parallel testing of specimens with the Alinity m MPXV RUO test (Abbott Molecular Inc., USA) and the in-house assay (extraction: Nimbus, Seegene, Korea; primers and probes: TibMolbiol, Germany; PCR: Cfx96 system, Bio-Rad, Germany) to estimate the correlation between both tests. Assay linearity was assessed with a dilutional series prepared from a cell-culture supernatant (INSTAND e.V., Germany) quantified by digital PCR, with concentrations ranging from 500,000 copies/mL to 160 copies/mL and tested at 10 replicates each. Detection limits of both tests (95% hit-rates) were determined by Probit analysis.

RESULTS

All patient samples initially negative for MPXV with the in-house PCR test were confirmed as negative with both tests. 95.3% (286/300) of the diluted samples with initial inhouse PCR-positive results were reported positive with both tests. Samples that were not positively confirmed by both tests had Ct values of >37 cycles upon initial testing of undiluted material. The correlation between results of both tests was very high (R^2 0.96). The mean difference in Ct values determined by Bland-Altman analysis was 0.54 cycles, with the lower Ct values for the Alinity m MPXV RUO assay. The dilution series demonstrated linearity for both assays. Probit analyses showed 95% hit-rates of 293 copies/mL for the MPXV RUO assay and 446 copies/mL for the in-house assay.

CONCLUSIONS

In our comparative analysis, the Alinity m MPXV RUO assay showed very high specificity and sensitivity with a lower detection limit of 293 copies/mL at a 95% hit rate. Continuous random access and stat capabilities of the Alinity m system allowed for improving turn-around-time of results in comparison to the batch-based in-house assay.

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Emerging and re-emerging viruses

IDENTIFICATION OF SARS-COV-2 OMICRON BA.5 STRAINS WITH DELETIONS OF THE ORF8 GENE IN NASOPHARYNGEAL SWABS OF COVID-19 PATIENTS

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BACKGROUND-AIM

Epidemiological surveillance of SARS-CoV-2 infection is useful to identify new variants and mutations which might modify the viral fitness. SARS-CoV-2 ORF 8 is an accessory protein involved in the host's immune system modulation.

METHODS

Nasopahryngeal (NSP) swabs were collected from patients subjected to SARS-CoV-2 epidemiological surveillance in Lombardy, Italy, and tested for the presence of SARS-CoV-2 genome. The viral genome, when present, was subjected to Next Generation Sequencing (NGS), and the presence of the more frequent mutations was confirmed by specific PCR and subsequent sequencing by Sanger method. SARS- CoV-2 strains were isolated from NSP, on VERO E6 cells, titrated by plaque assay, and the kinetic of their replications was defined in VERO E6 and LLC cell models, in vitro, by measuring the viral load in cells supernatants, at different times, up to 7 days post infection.

RESULTS

In the period between 11 August and 4 November 2022, 170 NSP were collected. A deletion of SARS-CoV-2 ORF8 gene was found in 14/170 (8.24%) NSP, by NGS analysis. In all samples, this deletion was confirmed by PCR of ORF8 and by subsequent analysis of sequence, by Sanger method: 1/14 sample (7.15%) had a partial deletion of 226 nucleotides, while 12/14 (85.7%) had a complete deletion of 417 or 418 nucleotides and 1/14 (7.15%) had a complete deletion of 727 nucleotides. The deletion-free virus and the one with the complete ORF8 deletion were successfully isolated from NSP, titrated and used to perform in vitro infection kinetic analysis in the VERO E6 and LLC cell models. The kinetics, followed over time, up to 7 days post infection, showed no differences in terms of trend and viral load between the two variants of SARS-CoV-2, in both the cell lines.

CONCLUSIONS

Presence of ORF8 gene deletions was found in NSP collected in a limited time range (about three months) and had never been found again until now. The deletions did not modify the kinetic of viral replication in vitro. Probably, such deletions are negatively selected, since they do not they represented an evolutionary advantage for the virus itself. However, analysis of cytokines production are ongoing to verify the role of ORF8 in the host's immune system modulation.

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Emerging and re-emerging viruses

INDIRECT IMMUNOFLUORESCENCE TESTING OF IGM AGAINST SANDFLY FEVER VIRUS IN PATIENTS WITH EVIDENCE OF ACUTE NEUROLOGICAL DISEASE

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BACKGROUND-AIM

Sandfly fever is a viral disease transmitted to humans through various sandfly species (Phlebotominae) during blood sucking. Symptomatic sandfly fever virus (SFFV) infections typically present as a self-limiting flu-like illness, with only the SFFV species Toscana (TOSV) causing neurologic symptoms. The disease is widespread in the Middle East, Central Asia, Northern Africa and the Mediterranean, but climate change is predicted to expand its distribution into Central Europe. In this study, the serodiagnostic performance of an SFFV indirect immunofluorescence assay (IFA) was evaluated in patients with confirmed or suspected SFFV infection of the central nervous system (CNS).

METHODS

Samples were collected 2-24 days post symptom onset (dpso) from 31 Algerian patients whose clinical presentation was consistent with TOSV infection of the CNS (e.g., meningitis). In 15 cases, PCR was performed on cerebrospinal fluid collected 1-4 dpso, confirming infection with TOSV. Anti-TOSV IgM titers were determined using the Sandfly Fever Virus Mosaic 1 IFA (EUROIMMUN), which is based on cells separately infected with the SFFV species Sicilian, Naples, Toscana and Cyprus. Samples were tested starting from a dilution of 1:10.

RESULTS

Anti-TOSV IgM was detected at titers of ε1:10 in 26/31 (83.9%) of supposedly SFFV-infected patients, including 13 PCR-confirmed cases, while five were non-reactive. The highest median IgM titers (ε1:320) were observed within two weeks after symptom onset.

CONCLUSIONS

The Sandfly Fever Virus Mosaic 1 IFA enables sensitive and specific detection of anti-SFFV (TOSV) IgM antibodies, indicating the suitability of serological testing to support the diagnosis of sandfly fever in symptomatic patients.

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Emerging and re-emerging viruses

MONITORING THE GENOMIC EPIDEMIOLOGY OF SARS-COV-2 IN THE PANDEMIC PERIOD IN ISTANBUL, TURKEY

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BACKGROUND-AIM

In this study, it was aimed to reveal the distribution networks of SARS-CoV-2 in Istanbul by performing full-length genome sequencing and to monitor the changes in the virus genome.

METHODS

The study included 203 samples that were found to be positive for SARS-CoV-2 RNA, belonging to different time periods between 2020-2022. Next generation sequencing was performed using the Illumina MiniSeq device “high-output” sequencing kit. Bioinformatic analyzes of obtained sequences was made using Illumina SARS-Cov-2 NGS Data Tool kit and online www.genomedetective.com/app/typingtool/virus/ software. Also, aligned files of nucleotide sequences obtained, are added to both Nextstrain (https://clades.nextstrain.org/)
as well as the GISAID (https://www.gisaid.org/) database.

RESULTS

In the clade analysis of the 203 SARS-CoV-2 RNA positive samples sequenced; 19A, 19B; 20A, which has spread rapidly in Europe since March 2020 and dominated the first wave of the epidemic; 20B, 20D evolving from 20A; 20I (Alpha, V1), 20H (Beta, V2), 20J (Gamma,V3), 21D(Eta) the variant of concerns (VOCs) responsible for the next waves and their subgroups; 21J (Delta), responsible for the fourth wave; 21K (Omicron), 21L (Omicron), and 22B (Omicron) groups were detected.

CONCLUSIONS

As a result, although it has been shown that different viral origins are circulating in our territory, it is important to detect the mutations affecting the pathogenicity and the changes in the primer target regions published in different databases in terms of evaluating the clinical courses and the performance of the molecular diagnostic kits.

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Emerging and re-emerging viruses

MONKEYPOX CASES AMONG CISGENDER WOMEN: FEATURES OF 5 MONKEYPOX CASES IN THE TOULOUSE AREA, MAY TO AUGUST 2022

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BACKGROUND-AIM

While accounting for less than 4% of cases, the spread of Monkeypox (Mpox) virus to women is of concern, particularly due to the risk of serious consequences for fetuses if pregnant women become infected. Clinical characteristics and transmission routes have been extensively characterized in men having sex with men (MSM), but few data exist on Mpox infections in cisgender women.
The aim of this study was to assess the clinical features and potential routes of transmission of Mpox cases among cisgender women, whose virological analyses were performed at the Toulouse University Hospital.

METHODS

Demographic, clinical, and therapeutic data of all PCR-proven Mpox cases among cisgender women in the Occitanie region between May 25th and August 31st,2022 were collected using an anonymized questionnaire.

RESULTS

We report five Mpox infections in cisgender women, aged 19 to 43 years old. All patients reported having sex with men. None were pregnant, immunocompromised, PrEP users, drug users or sex workers. Clinicians ascribed the route of transmission as sexual contact in 4 patients, 2 of whom returned from a trip (Spain, Italy) within the last week before the onset of the infection. An occupational route of transmission was suspected in one patient who worked as a medical laboratory technician. Confirmed or suspected sexually transmitted Mpox infected women presented pustular rash (n=4) along with mucosal lesions involving the vagina (n=4), anus (n=3) and oro-pharynx (n=1). These patients had inguinal adenopathy (n=2), dyspareunia and dysuria (n=2), leucorrhea (n=1), and pharyngitis (n=1). The patient with a suspected occupational contamination initially presented with a localized rash on the right wrist, then spread to the limbs and torso, while the anogenital area remained free of lesions. Two patients developed skin superinfection; no deaths were reported, no specific antiviral treatments were administered.

CONCLUSIONS

Although rare (1.89% of total PCR-confirmed Mpox cases in Occitanie), the diagnosis of Mpox infection should be considered in cis-gender women, even in the absence of risk factors or sexual exposure. Original transmission routes, as household and occupational exposure, are more common in cisgender women, unlike MSM and transgender women.

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MONKEYPOX VIRUS OUTBREAK IN LOMBARDY, NORTHERN ITALY 2022

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BACKGROUND-AIM

Since May 2022, Monkeypox virus (MPXV) has been responsible for a global outbreak, the first related to Orthopoxvirus (OPXV) after Smallpox virus eradication, with more than 86,746 cases and 112 fatalities worldwide, the majority of which in countries where MPXV was never reported before. Until April 12th, 2023, European Union (EU) accounted for 25,874 cases, with a hospitalization rate of 6%.
Lombardy, Northern Italy, is the most densely populated Italian region (10 million inhabitants) accounted for more than 40% of national MPVX cases. In this epidemiological scenario, the aim of this study was to describe clinical and virological data collected during the monitoring of 353 MPXV-positive subjects. In more details the aims of the study were: a) evaluate the presence and the shedding duration of MPXV DNA in different body compartments correlating the MPXV viability with the time to onset of symptoms. b) provide evidence of MPXV persistence in different body compartment as a source of infection and c) characterize the MPXV evolution by whole genome sequencing (WGS) during the outbreak occurred in Italy.

METHODS

The study included 353 patients with a laboratory-confirmed diagnosis of MPXV infection screened in several clinical specimens in the period May 24th - September 1st, 2022. Viral isolation was attempted from different biological matrices and complete genome sequencing was performed for 61 MPXV strains.

RESULTS

MPXV DNA detection was more frequent in the skin (94.4%) with the longest median time of viral clearance (16 days). The actively-replicating virus in cell culture was obtained for 123/377 (32.6%) samples with a significant higher viral quantity on isolation positive samples (20 vs 31,$p<0.001$). The phylogenetic analysis highlighted the high genetic identity of the MPXV strains collected, both globally and within the Lombardy region.

CONCLUSIONS

Skin lesion is gold standard material and the high viral load and the actively-replicating virus observed in genital sites confirms that sexual contact plays a key role in the viral transmission.

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Emerging and re-emerging viruses

MPOX VIRUS OUTBREAK IN PORTUGAL – REFERENCE LABORATORY FOR EMERGENCY RESPONSE EXPERIENCE

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BACKGROUND-AIM

Mpox is a zoonotic disease caused by mpox virus (MPXV). Since May 2022, several cases of mpox have been reported in different countries where the disease is not endemic. As of April 2023, 87.039 confirmed cases of mpox, including 120 deaths, have been reported by the World Health Organization in 110 countries. The aim of this study is to describe the results of laboratory diagnosis of mpox cases in Portugal.

METHODS

The laboratory diagnosis of mpox was based on the Real Time PCR method, due to its accuracy and sensitivity. Specimens were inactivated in a BSL-3 laboratory. The recommended specimens for laboratory confirmation of mpox were lesion and oropharyngeal swabs. Based on the patient's clinical presentation, other samples were considered for investigation, such as genital and/or rectal swab, urine and semen.

RESULTS

Portugal was one of the first countries to report cases of mpox, the first case being reported on May 16, 2022. To date, 953 cases have been laboratory confirmed and reported, mostly in the 30-39 age group (n= 420; 44.1%), in males (n=944; 99%) and mainly affecting men who have sex with men. There were only nine cases (1%) in females, two of them in pregnant women. Positive cases were detected in all regions of the country, but it was in Lisbon and Tagus Valley region (n=753; 79%) that the highest number was recorded. The MPXV detection was more frequent in lesion (n=986; 58.1%), oropharyngeal (n=596; 35.1%), and rectal swabs (n=75; 4.4%) and in urines (n=14; 0.8%). Lesion and rectal swabs showed lower mean values of Ct (cycle threshold), Ct=24 and Ct=25, respectively, suggestive of a higher viral load, compared to oropharyngeal swabs (Ct=30) and urines (Ct=29).

CONCLUSIONS

The Emergency Response and Biopreparedness Unit at the National Institute of Health, which is the reference laboratory for Orthopoxvirus, has implemented a laboratory algorithm that guarantees an accurate and quick response. This algorithm allowed the diagnosis of the first cases of mpox and is periodically evaluated by several European quality control systems, playing an essential role in the success of the national response to this outbreak.

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Emerging and re-emerging viruses

MULTIPLEX PCR APPROACH FOR SEVERE AND EMERGING RESPIRATORY INFECTIONS FOR PATIENTS HOSPITALIZED IN INTENSIVE CARE UNIT: ENTEROVIRUS/RHINOVIRUS UNDERESTIMATE ?

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BACKGROUND-AIM

Severe respiratory infections by SARS-CoV-2 are a major cause of morbidity and mortality worldwide, while a range of other respiratory pathogens can cause severe illness, in vulnerable individuals. Rapid and etiologic diagnosis of respiratory infections is crucial for effective clinical management, infection control, and public health surveillance, such as syndromic panel-based assays targeting several respiratory pathogens. The aims of the work were (i) to gather data on the spread of non-COVID-19 viruses during SARS-CoV-2 circulation, (ii) to study the interest of multiplex tests for respiratory infections diagnosis in the hospital, focusing on the intensive care unit (ICU).

METHODS

A retrospective study (Jan-2021_June-2022) analyzed 2,383 respiratory samples by multiplex PCR (FilmArray®/Biomérieux) : for samples from the upper respiratory tract (RP2.1, 19 viruses/4 bacteria) or for samples from the lower respiratory tract (Pneumonia FA-PP, 8 viruses/18 bacteria), with a SARS-CoV-2 PCR. For 18 patients from the ICU, viral data and the conclusions drawn by the clinicians were analyzed (Excel-Python).

RESULTS

The RP2.1 panel on 990 samples detected at least one virus on 450 samples (45.5%), including 41 viral co-infections: entero/rhinovirus (168, 37.3%) in co-infections (37, 8%), then SARS-CoV-2 (99, 22%). The Pneumonia panel on 1,393 samples detected at least one bacteria and/or one virus on 841 samples (60.4%), including 81 viruses alone (9.6%). For ICU patients, for whom at least one virus was detected on FA-PP: for 7/18 patients, the identification of the virus (i.e. influenza virus mainly) influenced the care management, while frequent entero/rhinovirus (8/18) were not clearly retained in the clinical conclusion.

CONCLUSIONS

Our results showed (i) the frequency of entero/rhinovirus (37.3%) with severe clinical forms, (ii) the complex co-circulation of respiratory tract viruses during COVID-19 spreading, (iii) that the impact of molecular diagnosis of respiratory infections on clinical practices, medical prognosis and economic weight deserves complemaentary studies.

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Emerging and re-emerging viruses

NATIONWIDE PREVALENCE OF MOSQUITO POPULATIONS AND GENETIC ANALYSIS OF FLAVIVIRUS IN THE REPUBLIC OF KOREA

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BACKGROUND-AIM

The Korea Disease Control and Prevention Agency has established regional centers to monitor population density and pathogens related with climate change since 2010. To monitor vector control measure and limit the probable impact of pathogens, it is significant to identify the timing of peak adult mosquito abundance to determine the efficiency for decreasing disease risks to human societies. Therefore, we studied the geographical and temporal distribution of mosquito populations, and epidemiology of flavivirus in mosquitoes in Republic of Korea.

METHODS

Mosquitoes were collected from CDC black-light traps and BG-Sentinel traps captured at 36 collection sites nationwide in 2020. The collections were performed at each location for a 24 hour period every two weeks (1st and 3rd week of every month) from March to November. Female mosquitoes were identified and confirmed morphologically by optical microscopic examination using taxonomic keys. Except anophlex, mosquitoes were used to detect flavivirus infection including West Nile virus, Zika virus, Japanese encephalitis, yellow fever virus, and dengue fever virus using qRT-PCR and phylogenetic analysis were performed.

RESULTS

67,203 mosquitoes were collected from traps, with a trap index of 36.6. The main mosquito incidence varied from May to October, and the highest peak in the population was observed in September. The predominant mosquito species were Culex pipiens, Armigeres subalbatus, Aedes albopictus, Aedes vexans and Culex tritaeniorhynchus. To monitor flavivirus, mosquitoes were pooled into 4,953 pools, and the minimum infection rate of flavivirus was 0.01%. Japanese encephalitis (JE) were only detected in seven pools of Culex orientalis from Sangju region, where habitats of migratory birds and we isolated virus from two pools. All the detected JE were revealed as genotype V by phylogenetic analysis.

CONCLUSIONS

To the best of our knowledge, this is the first study to isolate genotype V JVE from Culex orientalis in the Republic of Korea. Further geographical and ecological study on mosquitoes will improve our understanding of flavivirus risks. Thus, additional researches are needed to analyze the distribution of mosquito species and improved monitoring and long-term surveillance of flavivirus are of great public health im-portance.

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Emerging and re-emerging viruses

NATIONWIDE PREVALENCE OF TICK POPULATIONS AND GENETIC ANALYSIS OF SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME VIRUS IN THE REPUBLIC OF KOREA

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BACKGROUND-AIM

The Korea Disease Control and Prevention Agency has established regional centers to monitor population density and pathogens related with climate change since 2010. Due to the increasing number of severe fever with thrombocytopenia syndrome virus (SFTSV) infection in humans, where is a raised level of public health issue. So, we studied the temporal and geographical distribution of tick populations, and epidemiology of SFTSV in ticks in Republic of Korea.

METHODS

Ticks were collected from dry ice bait-collecting traps captured at 16 collection sites nationwide in 2020. The collections were performed at each location once a month, from April to November. Ticks were identified taxonomically and by life stage via optical microscopic examination using morphological keys. Approximately half of the collected ticks were used to detect SFTSV infection using RT-PCR and phylogenetic analysis were performed.

RESULTS

63,376 ticks were collected from traps, with a trap index of 41.3. The main tick incidence varied from April to October, and the highest peak in the population was observed nymph in May, adult in July, and larva in September. The predominant species were Haemaphysalis longicornis, Haemaphysalis sp., H. flava, Ixodes sp., Amblyomma testudinarium, and Ixodes nipponensis. To monitor SFTSV infection in ticks, approximately 50% of ticks were pooled into 2,973 pools, and the minimum infection rate (MIR) of SFTSV was 0.2%. Andong had the highest MIR for SFTSV (4.0%). The B3 genotype was most common (52.2%), followed by the B2 (28.6%), B5 (15.9%), B4 (1.6%), and B6 (1.6%) genotypes.

CONCLUSIONS

We reported that species diversity, species composition, abundance, and distribution of ticks and their pathogens to monitor and decrease the potential for autochthonous transmission of SFTSV. We recognized broadly distributed tick species and a high degree of diversity in SFTSV strains in ticks from different geographical regions. The result may offer the basis for upcoming epidemiological studies and risk assessment of tick-borne diseases.

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Emerging and re-emerging viruses

OPTIMIZATION OF MPOX PCR ASSAYS IN SINGAPORE GENERAL HOSPITAL, SINGAPORE

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BACKGROUND-AIM

Human Mpox virus (MPXV) is a double-stranded DNA virus of the Orthopoxvirus (OPXV) genus from Poxviridae family. During May 2022 outbreak, Singapore detected the first imported and first local case of Mpox on 20 June 2022 and 6 July 2022 respectively. As of April 2023, there have been 23 cases of Mpox reported in Singapore. This study is aimed to validate an in-house developed real-time PCR methods, which modified from previously published protocols from the US Centers for Disease Control and Prevention (CDC). MPXV generic PCR targeting both Clade I and Clade II of Mpox DNA (reported by Li Y et al, 2010) and OPXV PCR targeting DNA polymerase gene of Orthopoxvirus (reported by Reynolds MG et al, 2010) are used for this validation study.

METHODS

Two control measures were incorporated into the existing protocol for quality assurance monitoring purpose. A total of 11 clinical samples from suspected Mpox cases and 20 External Quality Assurance (EQA) samples were used in this study. The analytical specificity was determined by testing from a panel of reference isolates or known clinical samples. A quantitated Mpox virus genomic DNA (Slovenia Ex Gran Canaria) DNA was tested to establish the analytical sensitivity.

RESULTS

As there was no reference PCR test for all suspected clinical samples, except 2 samples were sent to the National Public Health Laboratory (NPHL), the final clinical diagnosis was used as the reference method. Nevertheless, all 20 EQA samples and 2 specimens that were sent to NPHL yield a 100% concordance. A total of 15 clinical samples containing viruses, 37 bacteria isolates and 17 fungal isolates were also tested. These samples were tested negative, which yield a negative concordance of 100%. Detection limit of the MPXV and OPXV PCR assays are 33 copies/reaction and 38 copies/reaction at 95% CI respectively.

CONCLUSIONS

The combination of MPXV generic and OPXV real-time PCRs offer a sensitive and specific assay for the detection of Mpox virus in clinical swab samples. As a tertiary hospital, it is important that Mpox virus exposure is identified early as it is critical for clinical management and implementation of infection control measures.

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Emerging and re-emerging viruses

PERFORMANCE OF NEW NUCLEOPROTEIN-BASED ELISAS FOR SERODIAGNOSIS OF ACUTE CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS INFECTIONS

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BACKGROUND-AIM

Crimean-Congo hemorrhagic fever virus (CCHFV) causes a highly contagious disease, that is transmitted by ticks and has high case-fatality rates in humans. It is circulating in many Asian and African countries, but also spreading to and within Europe. To cope better with future outbreaks of Crimean-Congo hemorrhagic fever (CCHF), the WHO has pointed out the need for development and validation of CCHF diagnostics, including serological assays. Here, we analyzed the performance of two new nucleoprotein-based Anti-CCHFV IgM and IgG ELISAs (EUROIMMUN).

METHODS

Both ELISAs were compared to the VectoCrimean-CHF-IgM and -IgG ELISAs (Vector-Best) using the CCHFV Mosaic 2 IgM and IgG indirect immunofluorescence assays (IFA, EUROIMMUN) as reference. Assay sensitivity was determined using 49 acute-phase serum samples from symptomatic CCHFV-infected patients. The assessment of specificity was based on sera from 30 negative control patients (symptomatic at-risk group), 30 healthy blood donors and 29 patients with hantavirus or sandfly fever virus infections. All samples originated from Turkey.

RESULTS

Sensitivity of the EUROIMMUN ELISAs (IgM: 98.0%, IgG: 47.1%) exceeded that of the Vector-Best ELISAs (IgM: 95.9%, IgG: 35.3%). Specificity for IgM was slightly higher using the EUROIMMUN ELISA (86.4% vs. 84.7%), while both IgG ELISAs yielded a specificity of 100%. Comparison of the quantitative results revealed a very strong positive correlation between both test systems (IgM: r=0.868, IgG: r=0.913), whereas the qualitative agreement was substantial for IgM (84.1%, κ=0.673) and IgG (94.9%, κ=0.791).

CONCLUSIONS

The new EUROIMMUN Anti-CCHFV ELISAs are standardized and easy-to-use tools that reliably support the identification of acute CCHF cases, making them suitable for laboratories involved in on-site outbreak support.

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Emerging and re-emerging viruses

PERFORMANCES OF THE MONKEYPOX R-GENE® ASSAY

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BACKGROUND-AIM

Mpox virus cases of infections have been recently reported from countries where the disease is not endemic, indicating the need of development of ready to use assays for the rapid and efficient detection before worldwide spread. In this context, bioMérieux developed the MONKEYPOX R-GENE® RUO assay and presents its analytical performances.

METHODS

All tests were performed using an inactivated Mpox viral culture quantified by dPCR(Stilla) at 9E+08cp/mL. Extraction and amplification of contrived samples (culture spiked into clinical lesion swabs) were done using EMAG® and ABI7500 Fast/QS5, CFX96 or LC480. Inclusivity and exclusivity were assessed in silico and in vitro using inactivated viral cultures, clinical samples, g-blocks and plasmids specific to the assay. The LoD was determined with serial dilutions of the inactivated viral culture Mpox into clinical samples. Its confirmation was done on 20 extraction replicates. Comparison of sensitivity performances was also performed versus two commercial kits iSolutionsTM MonkeyPox FullPlex qPCR(RUO) and PKampTM Monkeypox Virus RT-PCR RUO Kit V1.

RESULTS

A 100% inclusivity for all Mpox sequences analyzed as of October 2022 was reported. No cross-reaction was observed for smallpox, camelpox, HSV1, HSV2 and VZV viruses. Out of 93 sequences of cowpox virus, 9 had shown in silico cross-reaction that was confirmed in vitro on g-blocks, without significant impact, as cowpox is a zoonosis, rarely transmitted to humans and treatment of severe infection cases is common for both viruses. 19 and 20/20 replicates of the inactivated Mpox viral culture at 100 cp/mL were detected respectively on CFX96 and on ABI7500 Fast/QS5/LC480. Finally, 18/20 of the same replicates were detected with PKampTM Monkeypox Virus RT-PCR RUO Kit V1 assay and only 12/20 were detected with iSolutionsTM MonkeyPox FullPlex qPCR(RUO) assay.

CONCLUSIONS

The MONKEYPOX R-GENE® RUO PCR assay for the Mpox virus detection on patient's samples has shown good performance (sensitivity, inclusivity, exclusivity). Sensitivity was shown to be better when compared to other commercial assays The detection can be performed on various amplification platforms with an equivalent sensitivity, while the endogenous internal cellular control allows to control process including sampling quality.

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Emerging and re-emerging viruses

SARS-COV-2 NEUTRALIZING ANTIBODY RESPONSE IN HEALTHCARE WORKERS WHO HAD COVID -19 OR WERE VACCINATED WITH CORONAVAC ONE YEAR AGO

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BACKGROUND-AIM

In this study, it was aimed to investigate the SARS-CoV-2 neutralizing antibody (NAbs), Anti Nucleocapsid (Anti-N) and Anti Spike (Anti-S) levels among healthcare workers (HCW) who had COVID-19 or were vaccinated with CoronaVac this study, as well as the effect of booster doses in these groups at last 6 months.

METHODS

An analysis of cross-sectional data from a cohort study is presented. Of the 6050 HCW included in the study, 160 were randomly divided into two groups of 80 participants each. The two groups were: those who were diagnosed with COVID-19 a year ago (C-19) and those who received a double dose of CoronaVac a year ago (CV).
NAbs, Anti-N and Anti-S levels were measured in these participants. Antibody measurements were performed with Maglumi SARS-CoV-2 NAbs (Snibe Diagnostic, China), Elecsys® Anti-SARS-CoV-2 and Anti-SARS-CoV-2 S test(Roche Diagnostics International Ltd, Switzerland) respectively.
The booster vaccination status, gender, age, departments and occupation of participants were considered as independent variables. Pearson Correlation, Kruskal Wallis-H, Mann-Whitney U and Student's t test were used for the analysis. Significance level was accepted as $p<0.05$.

RESULTS

NAbs and Anti-S levels were found to be significantly higher in patients who had received two doses of the BioNTech booster vaccine in CV and C-19 groups ($p <0.001$). In the CV group NAbs levels were higher in males than in females ($p=0.036$). There was no significant difference across genders in the C-19 group ($p=0.643$). In the C-19 group, Anti-S levels were significantly higher in participants aged >50 years ($p=0.004$) whereas in the CV group a similar level of Anti-S was found in all age groups ($p=0.735$).
There was a significant increase in Anti-N levels in those receiving CoronaVac booster doses in both groups ($p<0.001$). A similar level of NAbs and Anti-N antibodies was found in all age groups ($p>0.05$). Anti-S and Anti-N levels were similar across gender groups ($p>0.05$).
There was a strong positive and statistically significant association between Anti-S and NAbs levels both in the VC group ($r_{pearson} =0.623$, $p<0.001$) and in CV-19 group ($r_{pearson} =0.706$, $p<0.001$).

CONCLUSIONS

Booster vaccination with BioNTech had an impact on NAbs levels. The effects of vaccines on antibody levels vary from person to person.

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Emerging and re-emerging viruses

SEROLOGICAL AND ADAPTIVE RESPONSE IN 2022 OUTBREAK MPOX CASES

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BACKGROUND-AIM

In 2022 an unprecedented outbreak of mpox cases induced scientists to investigate the dynamics of the immune response against monkeypox virus (MPXV) infection and methods for rapid and effective diagnosis. Our study aimed to describe serological and adaptive response in mpox cases by evaluating kinetics of specific IgA, IgM and IgG, the immune profile of T and B cells and the immunological memory.

METHODS

Fifteen patients with a laboratory-confirmed mpox were enrolled, a subset of these (n=4) was HIV+. Samples were tested at different time points after symptoms onset (SO). A home-made immunofluorescence assay was performed to detect specific IgA, IgM and IgG. The immunological memory and the activation/exhaustion of T and B cells were evaluated by flow cytometry. A subgroup of nine patients was analysed for the release of circulating cytokines involved in the inflammatory response.

RESULTS

Serological analysis showed higher IgA levels, compared to IgM and IgG, early after SO (T0; mean 1:82, 1:30 and 1:29, respectively), with a similar kinetic between IgA and IgM. Immunophenotype analysis showed a reduction in CD4+ T cells and an increase in CD8+ T cells in mpox patients; higher Effector Memory CD8+ T cells and Effector Memory-Expressing CD45RA CD8+ T cells were present in HIV+ subjects. Interestingly, HIV+ patients showed an expansion of Treg cells in early phases of the disease, normalizing over time. Considering the inflammatory cytokines, mpox cases presented a prominent T helper (Th) 2 response and a damped Th1 response, with higher levels of Th2-associated cytokines (IL-6 and IL-4).

CONCLUSIONS

Our data suggest the relevance of specific IgA testing early after SO as a possible marker for early diagnosis. Despite the small number of patients, the observation of a different maturation state of effector cells in HIV+ patients early after SO, together with the high levels of T-reg and the cytokine profile, lead us to better understand the role of mpox-HIV co-infection in the mechanisms of immuno-evasion. Nevertheless, the perturbation of adaptive cellular immunity by HIV infection in our cohort does not appear to have impaired a rapid and effective response to MPXV, probably due to all patients in this study being well-controlled HIV+ patients.

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Emerging and re-emerging viruses

SYNDROMIC MOLECULAR TESTING IN PATIENTS PRESENTING WITH CLINICAL FEATURES SUGGESTIVE OF MPOX

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BACKGROUND-AIM

Many cases identified during the multi-country MPox outbreak of Summer 2022 involved rash with lesions exhibiting atypical appearance and presentation. A diagnostic toolkit utilising MPox and subsequently Orthopox real time PCRs proved vital; user-defined test requests were the mainstay. We seek to adopt a syndromic-based approach for vesicular rash with appropriate clinical context to expedite diagnosis, treatment and appropriate patient management.

METHODS

Between 21st of May and 30th of November 2022 185 patients submitted 198 swabs to the Specialist Virology Centre at the Royal Infirmary of Edinburgh. Samples were swabbed from 16 body sites, with penile swabs being most frequent. Samples were sent from multiple sites across the East of Scotland. Additional real time PCR tests conducted were retrospectively analysed to establish a test panel and inform a syndromic approach.

RESULTS

Patients referring samples to the SVC in Edinburgh for MPox testing had an age range of 1 to 67 yr (median 30 yr) and were predominantly male 161/185 (87%) versus 24/185 (13%). Ages range of MPox positive patients was 20-65yr (median 38 yr). Of the 198 samples received, 1 was excluded on the basis that the sending patient elected to self-isolate and 3 were excluded based on patient age and clinical history. Their respective samples were excluded and not tested. 39/194 (20.1%) samples were positive for MPox and 155/194 (79.9%) samples were negative. 14 samples were tested only for MPox, 12 samples received additional testing for enterovirus and parechovirus and 1 was tested for measles; these were all negative. 55/155 MPox negative samples were positive for another pathogen: 15 HSV1, 12 HSV2, 14 VZV, 10 Treponema pallidum, 2 Neisseria gonorrhoeae, and 1 Chlamydia trachomatis. 1 patient tested positive for HSV1 and LGV and an additional patient was given a clinical diagnosis of staphylococcus aureus folliculitis. One MPox positive patient also tested HSV 2 positive.

CONCLUSIONS

The 5 most appropriate candidates for this syndromic testing approach are: MPox, VZV, HSV1, HSV2 and Treponema pallidum. These data show that employing this novel panel-based approach regarding vesicular rash with suitable clinical history may be invaluable in the molecular diagnostic arena.

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Emerging and re-emerging viruses

TEST SENSITIVITY AND POTENTIAL IMPACT OF CIRCULATING VARIANTS – DATA FROM QCMD SARS-COV-2 ANTIGEN TESTING EXTERNAL QUALITY ASSESSMENT (EQA), 2021 - 2023

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BACKGROUND-AIM

While nucleic acid amplification tests (NAATs) remain the gold standard for clinical diagnostic detection of SARS-CoV-2 due to the high sensitivity and specificity, population testing with antigen tests has become a central part of testing policies for COVID-19 control and surveillance. Several regulatory-cleared tests are commercially available. However, their performance has been evaluated with early-pandemic variants, and continuous post-market review is consequently needed to determine whether detection rates of tests might be impacted by current variants or emerging variants of SARS-CoV-2.

METHODS

Since May 2021, several international EQA schemes (currently 8 challenges in total) have been distributed by Quality Control for Molecular Diagnostics (QCMD, Glasgow, Scotland, UK), containing inactivated SARS-CoV-2-positive samples of various genetic strains (including variants of concern, VOCs: e.g., Delta, Omicron BA.1, BA.2, BA.4, BA.5) at different concentrations and negative samples, for performance assessment of both laboratory-based immunoassays as well as those used within the Point-of-Care setting (PoC) such as rapid lateral flow antigen tests and PoC analysers. The primary EQA objectives are to assess the sensitivity of antigen tests and the potential impact of circulating SARS-CoV-2 strains on their performance.

RESULTS

All core samples were correctly reported by 76.1 to 100% at participant level and by 73.5 to 100% at dataset level within the different challenges. Sensitivity differences could be shown in viral loads and SARS-CoV-2 strains/variants including the impact on performance by a B.1.1.7-like mutant strain with a deletion in the nucleoprotein gene.

CONCLUSIONS

EQA schemes provide valuable data to inform participants about weaknesses in their testing process or methods and support ongoing independent assay evaluations for regulatory approval or post-market surveillance. As professional testing/surveillance (based on laboratory-based and point-of-care test) remains for infection management and measuring transmissibility, the performance of COVID-19 diagnostic antigen-based testing should be continuously monitored to ensure their usefulness.

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Emerging and re-emerging viruses

UNDERSTANDING AND PREDICATING EVOLUTION OF SARS-COV-2

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BACKGROUND-AIM

A key characteristic of all viral outbreaks is the evolutionary change of the viral sequence over time. During the SARS-CoV-2 pandemic, evolutionary changes have corresponded to flare-ups of infection as the virus has repeatedly adapted to hosts, evaded the immune system, and displayed partial vaccine escape. Understanding and quantifying constraints on the evolution of viral proteins has valuable applications for the design of vaccines and antivirals in addition to providing fundamental understanding of how the protein may evolve in response to new selective pressure. We have previously developed an amino acid substitution model using SARS-CoV-2 spike protein sequence and structure as inputs. The model works on the level of individual amino acids and combines existing predictive methods to probabilistically assess the likelihood of specific substitutions. We now try to understand how the model performs over evolutionary time and how quickly predictive power is lost when applied to substitutions on proteins that are evolutionarily distinct from input structure

METHODS

To accomplish this, we test the model using high quality spike structures of major SARS-CoV-2 variants as inputs. We assess how these input structures affect model performance for cladistically or temporally filtered validation datasets of SARS-CoV-2 sequences. For example, we compare the performance of the model using wildtype and Omicron input structures for substitutions occurring in the context of the Omicron variant spike protein.

RESULTS

The result of this project is new understanding of the limits of viral protein substitution models with potential to apply these methods to a wide range of other viruses.

CONCLUSIONS

The result of this project is new understanding of the limits of viral protein substitution models with potential to apply these methods to a wide range of other viruses.

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Enteroviruses

ECHOVIRUS 9 IS THE MOST PREVALENT ENTEROVIRUS IN 2022, BELGIUM.

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BACKGROUND-AIM

While most infections are asymptomatic, enteroviruses have been associated with a wide spectrum of both common and uncommon illnesses. Aseptic meningitis is the most commonly encountered illness associated with enterovirus infections and mainly affects very young children. Since 2014, Enterovirus D68 (EVD68) is emerging worldwide and is receiving high attention of public health authorities because of its magnitude and clinical presentation. Acute flaccid paralyse (AFP) in children under 15 years is the golden standard for polio detection.

METHODS

In the context of epidemiological surveillance in Belgium, a selection of EV-positive samples received by the national reference centre (NRC) at UZ/KU Leuven, were genotyped. Molecular typing was done by RT-PCR using different primer sets. Enterovirus species A and B were typed by sequencing part of VP1, while for species C and D, the VP4/VP2 region and the non-coding region were used.

RESULTS

In 2022, 4794 samples were analysed in the context of enterovirus detection, originating from 45 Belgian laboratories (with for some >one site participating). Of those, 712 (14.9%) samples, were found to be enterovirus positive, originating from 593 cases. Overall, 466 samples were genotyped, of which the majority were respiratory samples (n=358 or 76.8%). Rhinovirus A (RVA) was the most prevalent respiratory enterovirus (46.9%) followed by RVC (35.2%). EVD68 was only detected sporadically this year (1.1%). All positive samples in cerebrospinal fluid were genotyped (n=88) with echovirus 9 (44.3%) found to be most prevalent. Also all faeces samples were typed (n=15), where coxsackievirus A4 (26.7%) and B4 (26.7%) were most abundant.

CONCLUSIONS

Echovirus 9 was the most abundant enterovirus genotype in cerebrospinal fluid in the year 2022.
In respiratory samples, RVA was the most prevalent enterovirus and EVD68 was detected sporadically.
Acute Flaccid paralysis was reported in two cases this year, one was positive for coxsackievirus A4. No poliovirus was detected in Belgium.

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Enteroviruses

EARLY MOLECULAR EVOLUTION TRACKING OF EMERGING ENTEROVIRUS D68 BY COMPLETE GENOME SEQUENCING

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BACKGROUND-AIM

EV-D68 causes respiratory illness and, occasionally, neurological syndromes. Phylogenetic analysis is usually based on sequencing of VP1 gene. NGS technologies can allow a deeper analysis of the complete genome to identify molecular patterns eventually associated with severe respiratory or neurological syndromes. This study aimed at: I) designing a new PCR-based NGS method for EV-D68 whole genome sequencing; II) evaluating EV-D68 evolution in the 2022-2023 winter season along the entire genome.

METHODS

Respiratory samples (June-October 2022) were screened for EV-D68. New primers were designed for whole genome amplification; EV-D68 complete genome was divided into three segments and each segment was then amplified. Genomic libraries were prepared and the sequences obtained were analyzed with the bioinformatic platform INSaFLU.

RESULTS

A total of 578 samples were screened for the presence of EV-D68 and 49 (8.5%) were positive. Primers were designed using 906 EV-D68 complete genomes collected from online repositories. Sequences were sliced into 33-nt strings and then filtered to remove ambiguous positions. Sequences were trimmed to a melting temperature of 60°C, discarding all those mapping on human RNA. EV-D68 genome was divided into three segments, overlapping by 334 and 344 nucleotides respectively. The most frequent primers mapping in the overlapping regions were selected. Full genome amplification was obtained for 46/49 samples (93.8%). NGS sequencing was successful for 29/49 (59.2%) positive samples; the mean depth of coverage was 6582X and the mean percentage of genome covered by at least 10-fold is 98.3%. All detected EV-D68 strains detected belonged to Clade B and share a nucleotide identity of 97.3%. 30/309 (9.7%) aminoacidic changes were observed in the VP1 protein and 12 (40%) of them occurred in BC-loop and DE-loop regions. These regions are those more exposed on the surface of viral capsid and the more susceptible to the selective pressure of the immune response.

CONCLUSIONS

In conclusion, we evaluated a new protocol for EV-D68 complete genome amplification that could be useful both for phylogenetic analysis of circulating strains and to track different pathogenicity markers potentially related to complications of clinical syndrome.

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Enteroviruses

ENTEROVIRUS D68 DISEASE BURDEN AND EPIDEMIOLOGY IN HOSPITAL-ADMITTED INFLUENZA-LIKE ILLNESS, VALENCIA REGION, SPAIN, 2014-2020 INFLUENZA SEASONS.

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BACKGROUND-AIM

Enterovirus D68 (EV-D68) has emerged as an agent of epidemic respiratory illness and acute flaccid myelitis in the paediatric population. The EV-D68 circulation mainly follows a biennial pattern upsurge, starting in 2014. Here, we describe the epidemiology of EV-D68 cases linked to emergency admissions for influenza-like illness (ILI) in the Valencia Region, Spain, between late 2014 and early 2020, and the genetic variability and phylogenetic relationships of the isolated strains.

METHODS

Prospective hospital-based active surveillance study on respiratory infections. ILI cases were screened in 4-10 tertiary-care hospitals covering 22%-48% of inhabitants of our region. Swabs were tested by multiplex RT-PCR and the VP1 gene of EV-D68 positive samples was sequenced. Phylogenetic analysis and characterization of variants at antigenic sites was also performed.

RESULTS

Respiratory virus screening identified 68 EV-D68 positives, during seven consecutive surveillance periods. The temporal distribution was as follows: 2014 (13/235, 5.5% of the EV/RV positives), 2015 (2/246, 8.1%), 2016 (1/249, 0.4%), 2018 (52/605, 8.6%). Phylogenetic reconstruction of 59 isolates, showed that VP1 sequences from 2014 clustered mainly in B2 (6/12), and B1 (5/12) subclades, but also in A2/D1 (1/12). Sequences from 2015 (n=1) and 2016 (n=1) belonged to B3 and A2/D1 subclades, respectively. Besides, almost all sequences from 2018 (42/45) belonged to A2/D3, and only 3/45 belonged to the B3 subclade. While B1 and B2 viruses were mainly detected in children (80% and 67% respectively), B3 viruses were equally distributed between children and adults, and A2/D1 and A2/D3 were observed only in adults.

CONCLUSIONS

Two EV-D68 epidemics linked to ILI hospitalized cases occurred in the Valencia Region during 2014-2020 influenza seasons, one in 2014 and another in 2018. The first one affected more children and males while the second one, adults and females. No neurological pathologies were observed among the patients, but most of them presented acute respiratory infections, with three fatal outcomes and one ICU admission. The EV-D68 A2/D3 strains circulating in our region in 2018 are associated with severe respiratory infection in adults, and were closed relatives to those concurrently circulating in Europe and worldwide.

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Enteroviruses

HOSPITAL ADMISSION WITH ENTEROVIRUS INFECTIONS IN THE CENTRAL NERVOUS SYSTEM (CNS) - DENMARK 2015-2022

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BACKGROUND-AIM

Enteroviruses (EV) are the causal agent for the majority of viral CNS-infections. However, the burden of diseases caused by these viruses is largely unknown. We aim to describe the incidence and trends in hospitalizations and deaths of patients presenting to hospital with neurological syndromes (meningitis, encephalitis, meningoencephalitis and acute flaccid paralysis or acute myelitis) and have been diagnosed with an enterovirus-related-ICD -10 diagnosis (DA80, DA850, DA870*, DA880).

METHODS

Our study period covers hospital admissions from 1. January 2015- 31. October 2022. We linked data from the National Patient Discharge Register and the Danish National Death Register using the unique person-identification number in the vital statistic registry (CPR) and performed descriptive analyses, estimated length of stay and identified deaths during hospitalization and up to 30 days after discharge.

RESULTS

From 1. January 2015- 31. October 2022 we identified 446 individual hospitalizations with neurological disease from enterovirus infection ranging from 6 admissions in 2020 to 131 in 2018. Most patients were children younger than 1 year (21.52%) and young adults 18-35 years (36.54%), the majority were male (56.95%) and most frequent diagnoses were enteroviral meningitis (84.98%). Median length of stay were 2 days (IQR: 1-3), and 3 days (IQR: 1-5) for children under 1 year. No deaths were recorded in patients hospitalized with neurological disease from enterovirus infection in the National Death Registry looking at the 30-day window from patients discharged with an CNS-enterovirus diagnosis.

CONCLUSIONS

The number of annual hospital admissions with neurological disease from enterovirus infection varies widely between the study years, but the demographic profile of the infected patients is unchanged between the years included in our study.
The National Patient Register of Denmark covers all hospital admissions, but neurological disease is only marked with enterovirus as a causal agent, if the treating physician makes the registration. This decision will often be based on results of microbiological tests and the local norms of coding and registration.
Further studies are needed to validate the use of enterovirus specific codes in the National Patient Register of Denmark.

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Enteroviruses

INCREASED DETECTION OF ENTEROVIRAL MENINGITIS FOLLOWING A REDUCTION IN COVID-19 CASES IN HOSPITALIZED PATIENTS, ISRAEL 2019-2022

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BACKGROUND-AIM

Outbreaks of enteroviral meningitis occur periodically and may lead to prolonged hospitalization with severe outcomes. During the COVID-19 pandemic, the number of enteroviral cases were relatively low, similar to other respiratory viruses during the pandemic. However, as COVID-19 cases declined in 2022, increased cases of enteroviral meningitis were observed in hospitalized patients in Israel.

METHODS

CSF samples were collected from January 2019 to December 2022 from 5,244 patients, with 305 specimens detected as positive for enterovirus (5.8%). The positive samples were sequenced using Sanger sequencing, and phylogenetic trees of the dominant subtypes were constructed.

RESULTS

In 2019, 7.5% of the CSF samples tested positive for enterovirus. During 2020-2021 the percentage of enteroviral meningitis cases decreased by 50% (3.6% in 2020 and 3.3% in 2021) alongside the emergence of COVID-19 worldwide. As COVID-19 cases declined in 2022, CSF samples positive for enterovirus increased. Positive samples in 2022 were the highest in the 2019-2022 period (9.2%), and significantly higher than 2020-2021 ($p<0.05$). The small endemic outbreaks of enteroviral meningitis during 2022 were dominated by different enteroviruses, the main types detected were Echovirus 4 (14.6%), Echovirus 6 (21.3%) and Coxsackievirus B2 (CV-B2) (10.7%). Moreover, CV-B2 was accompanied by distinct features of encephalitis, ataxia and prolonged hospitalization. Interestingly, the surge in enteroviral meningitis occurred offseason and across all age groups.

CONCLUSIONS

An upsurge of enterovirus infection associated with meningitis was detected in 2022 after a decline in COVID-19 cases. The subtypes that increased during this period -19 were Echovirus-4, Echoviru-6 and Coxsackievirus B2, with unique symptoms exhibited among patients. Herein, we suggest the effects of social distancing and possibly viral interference are responsible for the results shown.

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Enteroviruses

NON-POLIOVIRUS ENTEROVIRUS SURVEILLANCE IN THE UK: ARE WE DETECTING AN INCREASE IN MORE UNUSUAL ENTEROVIRUS SPECIE C?

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BACKGROUND-AIM

Non-polio Enteroviruses (NPEVs) are common pathogens associated with a broad spectrum of clinical disease in humans, from asymptomatic infections to more serious such as meningitis, and acute flaccid paralysis. Genotyping of NPEVs associated with specific symptomatic presentations is important for patient management, outbreak investigations, monitoring virus evolution and development of public health strategies for enterovirus-associated diseases.

METHODS

Human NPEVs can be classified into four species, A to D and further divided into genotypes based on the sequence of the VP1 capsid gene. In the UK, laboratories voluntarily submit samples that have been tested positive for enterovirus (EV) for further typing and characterisation to the Enteric Virus Unit-UK Health Security Agency (EVU-UKHSA). Sequencing methodologies include Sanger and amplicon-based next generation sequencing. Genotyping results were retrieved from UKHSA laboratory information system.

RESULTS

Data analysis suggests an increase in the number of samples typed as specie C strains since January to December 2022 (n=44) compared with similar period in 2018 (n=5) and 2019 (n=1). Further, up to March 2023, 14 samples have been typed as specie C, suggesting a consistent trend. Among those NPEVs strains detected are coxsackievirus (CV) A1, CVA19, CVA20, CVA22, CVA24, Enterovirus (EV) C99, EV C105, EV C109, EV C116 and EV C117.

CONCLUSIONS

Several factors may have contributed to the increase in the number of NPEV specie C typed in 2022-23. Changes in hospital testing strategies with implementation of syndromic multiplex assays including enterovirus (EV) and increased awareness of EV circulation due to detection of Poliovirus in London sewage. The impact of the COVID-19 pandemics and the non-pharmaceutical interventions on EV circulation is not well understood and may also play a role. We cannot discard the possibility that the increase in specie C detections reflects a true upsurge in specie C circulation.
The support of a surveillance programme for EV circulation is essential the closer we get to controlling poliovirus (PV) disease as part of the World Health Organization Polio Eradication Initiative. It has been hypothesised that in a PV-free world other specie C strains have the potential to evolve into a new polio-like virus.

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Enteroviruses

POLIO AND NON-POLIO ENTEROVIRUSES ENVIRONMENTAL SURVEILLANCE IN SPAIN, 2013-2022

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BACKGROUND-AIM

Environmental surveillance (ES) by testing wastewater (ww) samples has been a valuable tool for monitoring presence and circulation of viruses such as poliovirus (PV) or SARS-CoV-2. The Spanish National Polio Laboratory (SNPL) have implemented PV ES since 1999, following WHO recommended methods based on cell culture (CC). This study described the results obtained during a period of 10 years.

METHODS

A total of 159 samples were collected from 27 ww treatment plants in Madrid region (Canal Isabel II SA MP) between 2013 and 2022 (average population 6.5 million). Before 2020, samples were concentrated through filtration methods but during COVID-19 pandemic, the technique was changed to precipitation with 20% PEG. PV and non-polio enteroviruses (NPEV) detection was performed by isolation in RD/L20B CC and PCR detection. Amplification in 3'-VP1 region followed sequencing was used for EV genotyping.

RESULTS

PV was rule out in all samples both by CC and PCR; NPEV were detected in 90/159 (56.6%) samples, of which 30 (33.3%) were detected by CC and PCR and 60 (66.7%) only by PCR. During 2013-2019, 28/69 (40.6%) samples were EV-positive whereas detection rate increased to 62/90 (68.9%) with PEG-concentration. NPEV species A (21%), B (43%) and C (36%) were identified. Concurrent detections were found in 11 samples. Out of the 105 NPEV detected, 72 (68.6%) were fully genotyped. A total of 23 different EV types were characterized being the most prevalent E11, CVB3, E3, CVA4, CVA19, EV-C116, EV-A71, CVB4, CVA1, E6, E18 and CVA14.

CONCLUSIONS

Routine ES performed in the SNPL during the last 10 years confirmed the absence of PV in ww samples in Madrid, and also served to investigate the presence of other EV. Some of them are the same NPEV detected in clinical samples during the corresponding year in Spain: E6 in 2015, EV-A71 in 2016 and 2018, E18 in 2020 and CVB3, CVB4 or E11 in 2022. However, other EV such as CVA6, which is one of the most frequent type detected clinically each year, is practically undetectable in ww. Neither EV-D68, despite being the most prevalent in 2018, 2021 and 2022. On the other hand, NPEV-C are often detected in ww, but rarely in clinical samples. Finally, PEG-precipitation seems to be a more sensitive method than filtration for EV concentration in ww samples.

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Hepatitis/HIV

A PRISMA SYSTEMATIC REVIEW OF THE RISK FACTORS FOR HEPATOCELLULAR CARCINOMA SECONDARY TO HEPATITIS C GENOTYPE 3 (HCV-GT3)

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BACKGROUND-AIM

Hepatitis C (HCV) is a blood-borne virus (BBV) which globally affects around 79 million people and is associated with high morbidity and mortality. Chronic infection leads to cirrhosis in a large proportion of patients and often causes hepatocellular carcinoma (HCC) in people with cirrhosis. HCV has six genotypes (GT1-GT6) globally with GT3 accounting for 17.9% of all HCV infections. HCV-GT3 is unique as it responds the least to directly-acting antivirals and patients with GT3 infection are at increased risk of HCC even if they do not have cirrhosis.

METHODS

This systematic review aims to synthesise existing and published studies of risk factors for HCC secondary to HCV-GT3 and evaluate their strengths and limitations. We searched Web of Science, Medline, EMBASE and CENTRAL for publications reporting risk factors for HCC due to HCV-GT3 in all settings, 1946–2023.

RESULTS

4,144 records were identified from the four databases with 260 records removed as duplicates via manual reviewing. 3,884 records were screened with 3,514 excluded. 371 full texts were assessed for eligibility with seven studies were included for analysis.
Of the seven studies, three studies were retrospective case-control trials, two retrospective cohort studies, one a prospective cohort study and one a cross-sectional study design. All were based in hospital settings with four in Pakistan, two in South Korea and one in the United States. The total number of participants were 9,621 of which 167 developed HCC (1.7%).
A total of seven studies assessed demonstrated cirrhosis to be a risk factor for HCC secondary to HCV-GT3 followed by higher age (5), with two studies each showing male sex, high alpha feto-protein (AFP), DAA treatment and achievement of SVR as risk factors for developing HCC.

CONCLUSIONS

Although, studies have shown that HCV-GT3 infection is an independent risk factor for end-stage liver disease, HCC, and liver-related death, there is a lack of evidence in the literature for specific risk factors for HCC secondary to HCV-GT3. Only cirrhosis and age have demonstrated an association; however the number of studies is very small, and more research is required to investigate risk factors for HCC secondary to HCV-GT3.

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Hepatitis/HIV

ANALYSIS OF HIV SCREENING TEST DATA PERFORMED AT PUBLIC HEALTH CENTERS IN KOREA (2011-2021)

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BACKGROUND-AIM

Public health centers have provided free human immunodeficiency virus (HIV) screening tests for residents including socially vulnerable groups in Korea. This study was to investigate the current status and implication of HIV testing by analyzing the HIV test data performed at public health centers.

METHODS

This report analyzed the characteristics of individuals who underwent HIV screening across 255 public health centers from 2011 to 2021. The HIV screening test results were analyzed by gender, nationality, and the reason for the HIV test.

RESULTS

The average number of HIV tests conducted by public health centers between 2011 and 2019 was 443,609. During COVID-19 pandemic, the number of HIV tests performed in 2020 and 2021 was 178,653 and 104,621, a decrease of 59.7%, 76.4%, respectively, compared to the previous average number. Annually, more females underwent HIV testing than males. The main reasons for HIV tests were prenatal examinations (24.7%), followed by health checkups (27.0%), sexually transmitted disease (STD) checkups (20.9%), voluntary testing (10.1%), and anonymous testing (5.9%). The HIV positive rate was 2 to 17 times higher in the voluntary, anonymous, and foreign resident checkup groups than in other groups compared to the number of tests. The HIV positive rate of foreigners was 0.23% to 0.55%, which was about three times higher than that of Koreans (0.08-0.19%).

CONCLUSIONS

Anonymous, voluntary, and foreign resident checkups groups were found to have a high prevalence of HIV. Free HIV testing at public health centers seems to contribute greatly to HIV identification and needs to expand to foreigners and vulnerable groups.

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Hepatitis/HIV

AUDIT OF HIV-1 VIAL LOADS IN PATIENTS FOLLOWING TRANSITION OF ASSAY FROM ABBOTT REALTIME HIV-1TM TO ROCHE COBAS 8800TM PLATFORMS

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BACKGROUND-AIM

From 2017, the regional virology laboratory in Northern Ireland has offered RNA only HIV-1 viral load testing to all Health and Social Care Trusts in the region using an Abbott Realtime HIV-1 assay. After verification in May 2020, the laboratory adopted the Roche Cobas 8800 total nucleic acid extraction assay. Subsequently, following its role out in June 2020, Genito-Urinary Medicine (GUM) users in Belfast reported concerns regarding higher numbers of patient with low level viraemias than with the previous assay.

METHODS

A multi-cycle audit was therefore undertaken to detect differences in rates of virological blips and low-level viremias between assays, to identify and address any technical issues with the Cobas 8800 assay and reduce any proviral-DNA within the sample, which could contribute to spuriously detectable viral loads.

RESULTS

Verification and performance monitoring demonstrated close correlation between both assays (r 0.9381, p <0.0001) with significantly higher values obtained using the Cobas 8800 (p 0.0056), but within the half log pre-defined acceptance criteria. An initial review of 3375 patient viral loads across the two assays was performed and demonstrated a decrease in undetectable and increase in detectable viral loads δ 200IU/ml, with stable values >200IU/ml, raising concerns that pro-viral DNA had been detected by the Cobas 8800 assay. Therefore, alterations were made to centrifugation followed by centrifugation at the point of running the assay, which increased undetectable and decreased low level (δ200IU/ml) viral loads. Transfer to secondary tubes demonstrated no difference in viral loads obtained (p 0.05774).

CONCLUSIONS

Alterations to centrifugation speed, time and re-centrifugation at point of run, all led to an increase in undetectable viral loads and decreased loads in <70 and 70-200 IU/ml groups, while transferring samples from a primary to secondary tube had no demonstrable effect on viral loads.

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Hepatitis/HIV

CHANGING TREND OF HEPATITIS A VIRUS INFECTION: AN OBSERVATIONAL STUDY FROM A TERTIARY CARE LIVER INSTITUTE IN INDIA.

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BACKGROUND-AIM

Hepatitis A virus (HAV) is the most common cause of acute viral hepatitis (AVH) in children and is hyper endemic in India. It causes self-limiting illness and rarely acute liver failure (ALF). There is a definite shift in clinical presentation and age of occurrence in the last decade. The present study was conducted to find out the incidence, clinical presentation and outcome of HAV infection in different age groups.

METHODS

Out of 14,807 requests for anti-HAV IgM from the period January 2014 to December 2022 that were received, HAV IgM positivity was seen in 1144, 8%. Cases were grouped as: pediatric (<12 years), adolescent (12-18 years) and adults > 18 years. HAV RNA PCR and genotyping was done.

RESULTS

Symptomatic cases requiring hospitalization were 690(60%) ,out of which 212(31%) were pediatric,174(25%) adolescent and 304(44%) adult cases. AVH occurrence was seen equal amongst all the groups: 146, 69% pediatric; 117, 67% adolescent and 207, 68% adults (p value = 0.43). HAV related ALF was also equal in adults 97,32% ;57,33% adolescent and 66,31% in pediatric (p value 0.8).Mortality was more in the adolescent group (17,10%) than seen in pediatric(10,5%) and in adults (18,6%) (p value = 0.01). HAV IgM positivity in pediatric age group was 35.5(23-54)% ,adolescents 26.8(16-40)% while in adults it was 37.4(19-57)% and showed a rising trend of positivity on year wise comparison. HAV infection was predominantly seen during the post-monsoon season (June to September) across all the age groups. Out of all the samples that were sequenced in 100% it was Genotype IIIa and similar across all age groups and clinical outcomes.

CONCLUSIONS

Rising trend of HAV infection seen in adults and adolescent population in India. HAV related ALF was associated with highest mortality in adolescent age group. In all the age groups infection were mostly seen during monsoon season.High degree of suspicion in adult ALF cases for HAV infection should be there and transplant options should be offered earlier.

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Hepatitis/HIV

COMPARISON BETWEEN AUTOMATED BATCH VERSUS NEW WALK AWAY HIV MONITORING SYSTEM

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BACKGROUND-AIM

HIV-1 viral load (VL) testing is recommended for the monitoring of antiretroviral treatment. In this study, we present the preliminary data of an ongoing comparison between the HIV1 ELITe MGB Kit - ELITe InGenius (ELITechGroup) and the Cobas HIV-1 test - Cobas 4800 system (Roche Diagnostics), for VL monitoring using plasma specimens from people living with HIV.

METHODS

We collected 144 plasma samples from HIV-1 positive patients under antiretroviral therapy enrolled at IRCCS S. Gerardo dei Tintori in Monza, Italy. Each sample was analysed on both Roche Cobas and ELITe InGenius assays and the results were grouped in detected, not detected (ND) and < lower limit of detection (LoD).
In correlation analysis, only the viral load data from samples who had quantitative values in both ELITe InGenius and Roche cobas were included for analysis. VL data were transformed into log 10 copies/mL and the correlation was determined by simple linear regression.

RESULTS

On Roche Cobas assay, 97 samples had detectable VLs while 27 samples returned ND result and the other 20 ones showed a result inferior to the LoD. On ELITe InGenius assay, 72 samples had detectable VLs while 47 samples returned ND result and the other 25 ones showed a result inferior to the LoD.
Overall, 61 plasma samples had quantifiable results on both ELITe InGenius and Roche Cobas platforms and a significant correlation was found with a slope of 0.9473 and R2 of 0.9215 (Figure 1).
The positive agreement was 91.8% (89/97), the negative agreement 40.4% (19/47) and the overall agreement 75% (108/144). The discordant rates between the two assays were 25% (36/144).

CONCLUSIONS

Correlation analysis with samples that tested quantifiable on both systems showed a good agreement: the high discordant rate is mainly due to samples tested <LoD or <50 cp/mL of VL on Roche system and ND on ELITechGroup system. Further investigations should be performed to evaluate the effect of different PCR targets and integrated HIV DNA.
The new walk away system from ELITechGroup could be a valid alternative where large batch processing systems are not required.

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Hepatitis/HIV

COMPARISON OF ELITE INGENIUS AND ALINITY M VIRAL LOAD ASSAYS FOR THE QUANTIFICATION OF HBV, HCV AND HIV-1 IN CLINICAL SAMPLES

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BACKGROUND-AIM

Viral load (VL) is fundamental to monitoring the efficacy of antiviral therapy, and pivotal in clinical decision-making in patients' management. The precision and accuracy of results are especially crucial at the clinical cut-offs defining therapeutic failure or adherence. This study compares the new HBV, HCV, and HIV-1 ELITe MGB kit® on the small foot-print integrated system ELITe InGenius® and HBV, HCV, and HIV-1 Alinity m. The analytical performances were evaluated also in relation to the clinically relevant cut-off.

METHODS

Between January 2021 and November 2022, 124 HBV, 120 HCV, and 113 HIV-1 clinical samples (plasma and serum) were prospectively collected under informed consent, as leftovers of the Alinity m (Abbott) routine test. On the Alinity m outcome, "undetectable" samples, and positives with VL between 1 to 7 log IU/ml for HBV and HCV, and 1 to 5 log copies/mL for HIV-1 were selected and stored at -80C° until processing with ELITe InGenius® (ElitechGroup S.p.A.). Results were ranked as "non-detected", "detected" (VL<LOQ or cut-off), and "quantifiable" (VL> LOQ or cut-off), and statistical analyses, were performed using the STATA SW v.16.0. AUSL Piacenza Ethics Committee approved the study.

RESULTS

In the HBV comparison, the median VL was 3.11 log IU/mL (interquartile range, IQR=1.81-3.99) by Alinity m, and 2.83 log IU/mL (IQR=1.67-4.18) by ELITe InGenius® and the correlation was high (R2= 0.97). The concordant results were 88.71% and the overall agreement was substantial (k=0.70; 95% CI=0.53-0.87).
In the HCV comparison, the median VL was 5.60 log IU/mL (IQR=2.08-6.40) by Alinity m and 5.20 log IU/mL (IQR=2.40-5.70) by ELITe InGenius® and the correlation was high (R2= 0.92). The concordant results were 89.16% and the overall agreement was substantial (‖ =0.76; 95% CI=0.59-0.94).
In the HIV-1 evaluation, the median VL was 2.42 log (IQR=1.73-3.91) by Alinity and 3.44 log (IQR=2.22-4.58) by ELITe InGenius® and the correlation was high (R2= 0.89). Considering the clinically relevant cut-off (50 cp/mL), the concordance was 87.61% and the agreement was substantial (k=0.67; 95%CI=0.52-0.82).

CONCLUSIONS

ELITe InGenius® was non-inferior to Alinity m in the VL monitoring of blood-borne viruses (BBVs) within the clinically relevant range.

081

Hepatitis/HIV

COMPARISON OF THE NEUMODX® AND ARTUS HBV QS-RGQ® ASSAYS FOR THE QUANTIFICATION HBV DNA IN CLINICAL SAMPLES

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BACKGROUND-AIM

Hepatitis B virus (HBV) is a major global health problem affecting more than 250 million people and causing nearly one million HBV-related human deaths annually. Plasma/serum HBV DNA load monitoring is essential to manage the disease and evaluate the treatment response. In this study, we aimed to compare the NeuMoDx HBV Quant Assay (NMD, QIAGEN GmbH) with the artus HBV QS-RGQ Assay (Artus, QIAGEN GmbH) using residual plasma samples and to evaluate the discordant results.

METHODS

The study included 200 patient plasma samples analyzed with the NMD assay in 2019-2020 and stored at -80°C in the Microbiology Laboratory of Dokuz Eylül University Hospital. Samples of 100 patients containing HBV DNA (32 samples between 100-1000 IU/ml, 33 samples between 1000-10000 IU/ml, 35 samples >10000 IU/ml) and 100 HBV DNA negative samples were retrospectively analyzed by artus in 2022. Discordant results were evaluated by cobas 6800 HBV DNA Test (Cobas, Roche Diagnostics)

RESULTS

Excellent agreement (ǀ : 0.92) was found between NMD and Artus tests. Of the 100 samples that were HBV DNA negative by NMD, 93 were negative and 7 were positive (<32 IU/ml) by Artus. All seven discordant samples were positive for HBsAg. With the Cobas test, 5 samples were positive (<34 IU/ml), one sample was negative and one sample was invalid due to insufficient volume. Of the 100 HBV DNA positive samples detected by NMD, 99 were positive and 1 was negative with the Artus assay. This sample was also HBV DNA negative by the Cobas test.
The difference between NMD and Artus results was ε0.5 log10 (0.55-1.65) in 23 samples. Cobas test used for discordant samples showed a better agreement with NMD results. In 12 samples with HBV DNA >10,000 IU/mL, there was a significant difference between HBV DNA log10 values determined by three different assays (p:0.002).

CONCLUSIONS

NMD and Artus HBV assays had comparable performance. Discordant results in quantitation were more frequent in samples with high viral load, however would not affect patient management. The use of frozen and retested samples may be the reason for the positive results below LLQ with Cobas and artus.

082

Hepatitis/HIV

COMPARISON OF TWO HDV RNA PCR ASSAYS - IS PERFORMANCE AFFECTED BY HDV GENOTYPE?

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BACKGROUND-AIM

In April 2021 we introduced reflex testing for Hepatitis D virus (HDV) antibody for all new hepatitis B surface antigen positive patients at Barts Health NHS Trust, London, UK. All HDV antibody positive samples are referred to an external laboratory for HDV RNA testing. To improve the diagnosis of HDV and turnaround times in our hospitals, we validated a commercial HDV RNA PCR kit. As part of this validation we retrospectively tested samples from HDV antibody positive patients with both negative and positive HDV RNA results reported by the external laboratory.

METHODS

We used the RealStar™ HDV RT-PCR Kit 1.0 (Altona Diagnostics) and compared to a locally developed assay at the external laboratory.

RESULTS

We tested 62 patient samples from 54 patients as part of the validation. In twelve samples HDV RNA was detected at the external laboratory and was also detected by the commercial assay (range 2.77 – 7.41 log10 IU/mL) with a mean 0.44 Log difference. For the 38 patient samples where HDV RNA was negative at the external laboratory, eleven samples (from nine patients) were found to be viraemic in our testing (range 0.95-4.50 log10 IU/mL). Five of these patients had normal liver function tests. For two patients no further information was available.

CONCLUSIONS

The discordant results obtained in eleven samples may reflect differences in assay sensitivity or specificity. The locally developed assay was introduced approximately 15 years previously when fewer complete HDV sequences for different HDV genotypes were available. Sample degradation may also explain some differences due to delays in refrigeration and transportation to the external laboratory. We are unable to confirm whether these discordant results are due to genotype as HDV sequencing is currently unavailable in the UK, however we are planning to introduce this at Barts Health later this year.

083

Hepatitis/HIV

EVALUATION OF A NOVEL FOURTH-GENERATION PROTOTYPE ASSAY FOR THE DETECTION OF HIV INFECTION ON VIDAS® INSTRUMENTS

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BACKGROUND-AIM

The new VIDAS® HIV DUO AG/AB prototype uses robust and high-performance raw materials that allow a run time reduction of at least 30 minutes compared to the current VIDAS® HIV DUO Ultra. The aim of this study was to evaluate the performance of this new combination assay compared to ARCHITECT HIV Ag/Ab Combo (Abbott), on various HIV genotypes.

METHODS

The principle of the test combines two automated reactions using the ELFA technique (Enzyme Linked Fluorescent Assay) and enables the simultaneous detection of p24 antigen and of anti-HIV-1 (groups M and O) and anti-HIV-2 total immunoglobulins using a double-sandwich EIA. The p24 antigen detection of HIV-1 variants is based on the use of a special cocktail of selected mouse monoclonal antibodies and on the labelling of the conjugate with an innovative patented chemical molecule, tetrabiotin, to enhance sensitivity. The total anti-HIV antibodies are detected with a HIV-1 recombinant envelope glycoprotein gp160 and HIV-1 group O/HIV-2 specific synthetic peptides. The biotin labeled conjugates are revealed through streptavidin coupled to alkaline phosphatase. At the end of the assay, antigen/antibody separate test values are generated, compared to the thresholds, and interpreted by the instrument.

RESULTS

The diagnostic sensitivity evaluated on 66 confirmed HIV-positive samples (41 HIV-1 group M, 10 HIV-1 group O, 10 HIV-2 and 5 seroconversion samples) was 100%. All 10 diluted HIV cell-culture supernatants representing different unusual HIV-1 subtypes were detected with higher test values with the VIDAS prototype than ARCHITECT HIV Ag/Ab Combo. The diagnostic specificity was 99.4% [98.3; 99.9]% determined on 500 hospitalized or high-risk behavior patients. Fifty-one HIV negative samples from patients whose physiological status may interfere with immunoassays, were tested: only 3 samples were obtained falsely positive with the VIDAS prototype against 9 with ARCHITECT HIV Ag/Ab Combo.

CONCLUSIONS

This study demonstrates very good performances of the newly VIDAS® HIV DUO AG/AB prototype in terms of sensitivity as well as specificity. This promising test will thus contribute to early, rapid, and sensitive detection of HIV infection which is essential for the management of the HIV and AIDS epidemics.

084

Hepatitis/HIV

EVALUATION OF DRIED BLOOD SPOT SAMPLES AS AN ALTERNATIVE TO SERUM FOR THE SEROLOGICAL DETECTION OF ANTIBODIES TO HIV, HEPATITIS C, HEPATITIS B AND SYPHILIS

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BACKGROUND-AIM

Assess whether dried blood spot samples (DBS) can be used as an equivalent alternative to serum for the detection of antibodies to HIV, hepatitis C, hepatitis B and syphilis for surveillance in marginalized and hard to reach populations.

METHODS

Study recruits from Specialist clinics, known to be positive for one or more of these infectious agents, provided a tube of EDTA blood from which DBS cards were prepared with varying amounts of whole blood. The remaining plasma from each recruit was tested to determine the S/Co (signal/cutoff) values for the respective analyte(s). The DBS spots were punched into a sterile container with a 12.7 mm diameter punch and eluted with Alinity wash buffer for 60 minutes with rotation at room temperature. Eluates were tested on the Abbott Alinity serology platform for antibodies to one or more infectious agent(s) based upon the clinic location of the participant. Analyte S/Co values from the eluate were compared with the respective plasma value and result interpretations.

RESULTS

The data from 44 study recruits tested when comparing concordance between the plasma and DBS results and interpretations was 100% for syphilis (24/24), HIV (19/19), and hepatitis C (7/7) respectively. For hepatitis B concordance was 89% (8/9) based upon a positive confirmation.
Testing the positive syphilis eluates to determine an RPR titre was found to be difficult due to the background haemolysis.

CONCLUSIONS

The results indicate that DBS samples are potentially a viable alternative to serum for the diagnosis of HIV, hepatitis C and hepatitis B.
For syphilis however, a positive result is an indication of potential exposure and would require follow up serology with RPR titres to assist with staging.

085

Hepatitis/HIV

FOUR-YEAR ANALYSIS OF GENETIC DIVERSITY AND HIV DRUG RESISTANCE MUTATIONS AMONG HIV-1 TREATMENT-NAIVE TURKISH COHORT

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BACKGROUND-AIM

In our study, we aimed to investigate transmitted drug resistance mutations (TDRMs), pre-treatment drug resistance mutations (PDRMs), and HIV-1 subtypes in treatment-naive patients diagnosed with HIV-1 infection and followed up in our center.

METHODS

275 treatment- naïve patients diagnosed with HIV-1 infection and followed up in Marmara University Hospital between 2017 and 2020 were included in the study. HIV-1 RNA level in plasma samples was studied by with COBAS, Ampliprep/COBAS TaqMan HIV-1 Test, v 2.0 kit (Roche, USA). Viral RNA isolation from plasma, RT-PCR, and DNA sequence analysis of RT and protease regions were performed with ViroSeq HIV-1 Genotyping System. Stanford HIVdb version 8.4 Genotypic Resistance software was used to analyze the mutations (Abbott/Celera Diagnostics, USA). Drug resistance mutations analysis and subtyping were determined using Stanford HIVdb v 8.6.1 Genotypic Resistance Software and TDRMs were determined according to the drug resistance surveillance list published by the WHO in 2009.

RESULTS

Remarkable clinical features of patients were as follows; subtype B, CDC category A, and CD4 count <350 were observed in 59.6%, 79.6%, and 50.2%, respectively. In naïve patients, the rate of PDRMs causing resistance in any class of antiretroviral drugs was found to be 22.2%, and the rate of TDRM was 7.3%. In the study, 4.0% and 3.3% of patients had NRTI and NNRTI TDRMs respectively whereas 8.7% and 16.0% NRTI- and NNRTI-PDRMs, but no major PI mutations were detected. The most frequent mutations were A62V (3,3%) and M41L (2,9%) in NRTI, E138A (9.5%), and K103N (2.2%) in NNRTI.

CONCLUSIONS

The rate of TDRMs is 7.20% which is consistent with the data of Turkey. The coexistence of M41L and T215D mutations are the most frequently detected TDRMs in the NRTI and the K103N mutation in the NNRTI group. E138A mutation causing NNRTI resistance is the most common mutation with a rate of 9.5%, and it is noteworthy that sixteen of the twenty-six patients carrying this mutation were B + CRF02_AG recombinant subtype. The rate of CRF and/or recombinant HIV-1 subtypes is 22.5%, with 15.3% of them, B+CRF02_AG is the most frequently detected CRFs subtype.

086

Hepatitis/HIV

HDV VIRAL LOAD QUANTIFICATION IN UNTREATED AND BULEVERTIDE-TREATED CHRONIC DELTA HEPATITIS PATIENTS IS SIGNIFICANTLY INFLUENCED BY RNA ISOLATION METHODS

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BACKGROUND-AIM

Diagnosis and management of Chronic Delta Hepatitis (CHD) requires highly sensitive and reliable tests for HDV RNA quantification. Aim of the study was to compare two different RNA isolation methods for HDV RNA quantification in untreated and Bulevertide (BLV)-treated CHD patients.

METHODS

96 frozen sera consecutive from 18 untreated and BLV-treated CHD patients were tested in a single-center retrospective study with RoboGene HDV RNA Quantification Kit 2.0 (Roboscreen GmbH, Germany; pangenotypic; LOD 6 IU/mL using ABI 7500 Fast (Applied Biosystem, Germany)) by using two methods: manual method, INSTANT Virus RNA/DNA kit (Roboscreen GmbH, Germany) vs. automated method, EZ1 Advanced XL instrument with EZ1 DSP Virus Kit (Qiagen, Germany). Analysis of HDV genotype was performed by sequencing of the Hepatitis Delta antigen region.

RESULTS

Main demographic, biochemical and virologic features of the patients analysed were: age 48 (29-77), 67% males, 100% caucasian and HDV genotype 1, 100% cirrhotics, all under NUC treatment, ALT 106 (32-222) U/L, AST 92 (52-214) U/L, HBsAg 3.7 (2.5-4.3) Log IU/mL, 94% HBeAg negative, 78% HBV DNA undetectable. Overall, HDV RNA was 3.61 (0.70-6.60) vs 2.66 (0.70-5.52) Log IU/mL by automated vs. manual method ($p<0.0001$). Compared to the automated method, manual method reported higher HDV RNA levels in 90 samples [median 0.80 (0.07-2.11) Log IU/mL], similar levels in 3 [two <LOD samples, and one 197 IU/mL], and lower levels in 3 [0.11 (0.09-0.17 Log IU/mL]. Among 18 BLV-treated patients (timepoints: baseline, week 4, week 8, week 16, week 24), virological response rates differed according to the RNA isolation method: manual method [baseline 4.89; 4.16; 3.52; 2.7; 2.26 Log IU/mL] and automated method [baseline 3.59; 3.41; 2.64; 1.61; 0.85 Log IU/mL], achieving <LOD HDV RNA in 11% vs 44% at week 24, respectively.

CONCLUSIONS

Quantification of HDV RNA in untreated and BLV-treated CHD patients by RoboGene kit 2.0 is significantly influenced by RNA isolation methods being manual extraction significantly more sensitive than the automated one by approximately 1 Log. RoboGene kit 2.0 is widely used in clinical trials with new anti-HDV therapeutics and correct HDV RNA quantification is significant to determine the effectiveness of therapy.

087

Hepatitis/HIV

HEPATITIS C AND THE "ICEBERG" EFFECT: EARLY DIAGNOSIS OF UNRECOGNIZED CASES TO ELIMINATION? THE HCV SCREENING CAMPAIGN EXPERIENCE IN PIACENZA

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BACKGROUND-AIM

Screening for antibodies and eventually for viral replication is considered essential to detect asymptomatic and unrecognized hepatitis C (HCV) infections (the "submerged part of the iceberg"), improving early diagnosis and interrupting the circulation of HCV. In February 2020 the Italian Parliament approved a two-year experimental screening of HCV infection aimed at all the population born between 1969 and 1989, subjects followed by public service for addiction, and individuals held in prison. The choice of the cohort derives from Italian epidemiology. Due to COVID-pandemic, the beginning of the screening was postponed to the end of 2021. Here we reported the experience at the AUSL of Piacenza.

METHODS

Anti-HCV antibodies (HCV-Ab) followed by quantification of viral replication (HCV-RNA) and genotyping (HCV-GT) were offered to the selected population. In the case of HCV-Ab positivity, HCV-RNA and HCV-GT were performed on the same sample. In our study, we examined data from free screening in the period between December 2021 and December 2022.

RESULTS

11049 people joined the HCV screening. 188 (1.64%) people resulted positive for HCV-Ab, despite being asymptomatic. All 188 serum samples were tested for quantification of HCV-RNA and 26 (0.23%) showed active viral replication (0,5x10^3 – 2.7x10^7 IU/mL). These people were invited to start therapy. HCV-GT was performed on 20 of 26 samples, due to insufficient sample volume or low viral load (<1x10^3 IU/mL).

CONCLUSIONS

The percentage of positivity highlighted in our geographical area is in line with data from previous studies (0.9%-2.3%). Our data confirm the usefulness of screening in identifying asymptomatic positive patients. Hopefully, the screening pool and interval will be expanded in the coming years, to improve the possibility of an early diagnosis, sending patients to treatment avoiding complications of advanced liver disease as well as to interrupt the circulation of the virus, and preventing new infections.

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Hepatitis/HIV

HIV SEROREVERSION OF HIV-EXPOSED UNINFECTED INFANTS IN KOREA, 2008-2021: A RETROSPECTIVE ANALYSIS

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BACKGROUND-AIM

In order to confirm HIV mother-to-child transmission in newborns, the Korea Disease Control and Prevention Agency (KDCA) has carried out periodic HIV tests for up to 18 months until residual maternal HIV antibodies have disappeared. This study aimed at identifying the mean duration of clearance of maternal HIV antibodies through the HIV infant test results from 2008 to 2021.

METHODS

For this HIV early infant diagnosis (EID), we have applied three types of HIV test; HIV virologic assays (DNA PCR and RNA viral load), HIV antigen (ELISA) assay, and antibody (ELISA and western-blot) assays; using the peripheral blood mononuclear cells (PBMCs) and plasma obtained from whole blood. The main analysis factors are the number of EID testing cases by year, the average duration of maternal HIV antibody loss (seroreversion) from positive to negative in infants.

RESULTS

The 108 infants (total 330 specimens, average 3.1 replicates) were tested to confirm vertical infection over 14 years period. Two cases were positive and 37 were finally reported as negative. Sixty-nine cases were stopped in the middle of the pending (undetermined HIV; no viral load but still has antibodies) status. Analysis of the 37 reported final negative results showed that the average time to transition from positive to indeterminate on western-blot assay was 11.3 months. The average duration of disappearance of maternal HIV antibodies was 14.9 months in the ELISA test, and it took 15.6 months after birth for the final negative results to be notified. Two cases reported as final positive were continuously positive in both HIV RNA and DNA from the initial samples.

CONCLUSIONS

This is the first analysis report on HIV-exposed infant tests in Korea, and can be used as basic data for the HIV vertical infection test algorithm. There have been no reports of Mother-to-child transmission in Koreans since 2014. Active follow-up of HIV-exposed infants to increase early diagnosis of HIV infection is critical as prompt initiation of antiretroviral therapy, and retention in care.

089

Hepatitis/HIV

SEROPREVALANCE AND RISK FACTORS FOR HEPATITIS B INFECTION AMONG PREGNANT WOMAN ATTENDING ANTENATAL CLINICS IN MOGADISHU, SOMALIA

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BACKGROUND-AIM

Hepatitis is a common term for liver inflammation caused on by a number of viruses, including Hepatitis A, B, C, D, and E. Hepatitis B virus infected more than 2 billion people alive today with 350 million infected chronically and being carriers of the virus. this study was carried out to determine the prevalence and risk factors of hepatitis B infection among pregnant women attending ANC clinics in Mogadishu, Somalia.

METHODS

The study was a cross sectional, the study subjects were selected by systematic random sampling and every fifth outpatient was included in the study and blood sample was taken for routine investigation. Blood samples were subjected to Hepatitis B screening by ELISA method after getting consent from the study subjects

RESULTS

Of the 384 pregnant women included in the study, 54 (14.1%) were sero-positive for Hepatitis B surface antigen. The significant risk factor for hepatitis positivity were female genital mutilation (COR-3.125; CI-95%1.089-8.96; p=0.0262), blood transfusion history (COR-3.54, CI-95%-1.01-7.79p=0.000135)and dental procedure history (COR-1.986; 95%CI- 1.11-3.54; p-0.0187). There was no significance difference with respect to positive history of jaundice (p= 0.432), and history of surgical procedure (p= 0.538).

CONCLUSIONS

The prevalence of hepatitis B was higher compared to the previous studies. Blood transfusion, history dental procedure and female genital mutilation were found to be associated with hepatitis B infection. Therefore creation of health awareness one mode of transmission is important.

090

Hepatitis/HIV

SEROPREVALENCE OF CMV, EBV AND HIV AMONG THE TURKISH PATIENTS

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BACKGROUND-AIM

Background: CMV and EBV co-infections can affect the HIV disease progression by modulating the immune system. In world, HIV is rapidly expanding. HIV is spreading rapidly around the world; however, it also causes other infections by affecting the immune system. In our study, we aimed to retrospectively determine the presence of CMV and EBV, which are frequently detected together with HIV.

METHODS

Method: Our study is a retrospective, single central observational study evaluating the data of the last 2 years. 78 patients were screened for CMV, EBV and HIV tests. DNA isolation and real-time PCR were performed with QiaSymphony and rotor gene device to detect CMV and EBV DNA, and HIV RNA PCR test was performed with GeneExpert device.

RESULTS

Results: Of 78 samples, the rate of active EBV and HIV co-infection in CMV-positive patient was observed in 55,1% (all of them are immunodeficiency) and 39,7% (n=8 transplant, n=11 leukemia), respectively. And, HIV and EBV were found together in 2 patients with non-Hodgkin lymphoma. In addition, CMV, EBV and HIV were detected together in 2 patients.

CONCLUSIONS

Conclusion: CMV-infection and HIV infection correlated with reactivation of EBV. We propose that these two viruses influenced the development and progression of the immunodeficiency and lymphoma.

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Hepatitis/HIV

TENOFOVIR RESISTANCE-ASSOCIATED MUTATIONS AT PATIENTS WITH CHRONIC HEPATITIS B

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BACKGROUND-AIM

Tenofovir is a nucleotide analog that is widely used for the treatment of HBV all over the world due to its high potency and high genetic barrier to resistance.
In this study, the presence of tS78T, rtA194T, and CYEI (S106C, H126Y, D134E, and L269I) and MLVV (rtL180M, rtT184L, rtA200V, and rtM204V) mutations associated with tenofovir resistance in the gene encoding the reverse transcriptase (RT) enzyme was investigated.

METHODS

Twenty treatment-naive patients who underwent liver biopsy due to chronic hepatitis B were included in this study. The gene region encoding the HBV reverse transcriptase (RT) enzyme was amplified by PCR and sanger sequencing was performed (Table 1)
To identify viral genotypes and mutations in HBV Pol/RT gene regions, www.ncbi.nlm.nih.gov/projects/genotyping and Geno2Pheno bioinformatics (https://hbv.geno2pheno.org/index.php) programs were used, respectively.

RESULTS

All (100%) HBV isolated from 20 patients with CHB were identified as genotype D
Tenofovir-associated mutations were detected in 6 patients (6/20, 30%). A194G mutations were detected in 1 (1/20, 5%) cases, and H126R mutations were detected in 4 (4/20, 25%) cases, and D134N mutations were detected 1 (1/20, 5%).No MLBB (rtL180M, rtT184L, rtA200V and rtM204V) mutations were detected in any patient (Table 2).

CONCLUSIONS

Identification of mutations related to potential TFD resistance in patients with treatment-naive chronic hepatitis B may contribute to more effective treatment strategies.

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Hepatitis/HIV

THREE-YEAR MULTICENTER ANALYSIS OF HIV INFECTION DIAGNOSTIC DATA

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BACKGROUND-AIM

In our country, the 3-stage national HIV testing algorithm was started to be used in HIV diagnosis with the HIV-AIDS Diagnosis Treatment Guideline updated in 2019. However, the performance of the algorithm in individuals receiving antiretroviral therapy (ART) early in infection or infected while receiving pre-exposure prophylaxis has not been comprehensively evaluated.
In this study, it was aimed to evaluate the effectiveness of the new algorithm as well as the changes in the HIV epidemic due to the limited access to health services caused by the Covid 19 pandemic.

METHODS

The test algorithms used in the diagnosis of HIV/AIDS infection, HIV test numbers, problems experienced in the field, and solution suggestions were evaluated with a 57-question survey of 29 HIV testing centers participating in the study. 12 centers participated with their HIV 1/2 Ab/Ag 4th generation ELISA and repeat reactive test numbers, distribution of confirmatory test results, and HIV NAT numbers for a total of 3 years between 2018-2021.

RESULTS

Between 2018 and 2020, the total number of Anti HIV ELISA tests and recurrent reactivity rates were 788261, 871299, 835498, and 0.48, 0.51, and 0.70, respectively, while the confirmed HIV test rates were 0.24, 0.25 and 0.21. The rates of Western Blot confirmatory test utilization in this period were 25.77%, 12.29%, and 9.86%, respectively, and it is seen that they have decreased gradually. The usage rates of HIV ½ Antibody differential rapid confirmatory tests recommended in the current algorithm were found to be 25.72%, 38.76%, and 45.78%, respectively, and it is seen that they have replaced WB. The rates of HIV RNA studied by year were 48.77%, 97.98%, and 46.31%.

CONCLUSIONS

In our study, it is seen that the use of the rapid HIV ½ antibody differential test included in the new algorithm is gradually increasing and is used more effectively in the diagnosis of HIV, while the use of HIV NAT has decreased. This situation, which is associated with the shift of NAT opportunities to Covid 19 PCR studies in the shadow of the Covid 19 pandemic, requires more detailed monitoring in terms of possible changes in the HIV epidemic in our country.

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Immune response and vaccines

AN INVESTIGATION INTO SARS-COV-2 IMMUNE RESPONSES AMONG VACCINATED AND UNVACCINATED HEALTHCARE WORKERS IN KANO, NIGERIA

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BACKGROUND-AIM

Immune responses to SARS-CoV-2 infection and vaccination are critical in combating the threat posed by the virus. Antibodies have been characterized to correlate with host protection against infection. Healthcare-workers represent one of the high-risk groups of contracting as well as spreading the disease, hence studying the burden of SARS-CoV-2 infection, as well as the immune response of this group to the virus is imperative. Therefore, this study evaluated SARS-CoV-2 antibody responses among vaccinated and unvaccinated healthcare-workers

METHODS

One hundred and sixty-four (164) healthcare workers consisting of 82 participants each for the vaccinated and unvaccinated group were recruited and assessed for their SARS-CoV-2 antibody response in relation to their sociodemographic characteristics, vaccination status, and their associated clinical features. The IgM and IgG were assessed using a rapid diagnostic test kit, while the neutralizing antibodies were assessed using enzyme-linked immunosorbent assay

RESULTS

The IgG and neutralizing antibodies responses were found to be significantly higher among the vaccinated group in comparison to the unvaccinated group. The overall prevalence of IgM and IgG antibodies among the vaccinated group were 1.20% and 95.0% respectively, and among the unvaccinated group were 3.70% and 84.10% respectively. The mean neutralizing antibodies titer was 165.77±10.90IU/ml and 129.62±10·7IU/ml for the vaccinated and unvaccinated group respectively

CONCLUSIONS

SARS-CoV-2 vaccination induces a robust antibody response and likely reduces the risk of SARS-CoV-2 infection; however, such response decreases with time and a booster vaccination may help in sustaining vaccine-induced immunity.

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ANTIBODY RESPOND DYNAMICS IN HEALTHCARE WORKERS AFTER SARS-COV-2 VACCINATION: A COHORT STUDY

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BACKGROUND-AIM

Vaccination played an important role in controlling the COVID-19 pandemic. Vaccine-induced antibody levels are strong indicators of protection against the disease.
In this prospective cohort study, we aimed to assess SARS-CoV-2 neutralizing antibody (NAb) production in vaccinated healthcare workers (HCWs) using a surrogate viral neutralization test (sVNT) and demonstrate the association of NAbs with SARS-CoV-2 anti-receptor binding domain (RBD) immunoglobulin G (IgG).

METHODS

Anti-RBD IgG and NAbs levels were measured at four different time points in 226 HCWs who had completed two doses of CoronaVac and received one or two booster doses of CoronaVac or BNT162b2. SARS-CoV-2 IgG II QUANT (Abbott, Chicago, IL, USA) and ACE2-RBD Neutralization Assay (Dia-Pro, Italy) kits were used in the study. Measurements were performed 1 and 4 months after two doses of CoronaVac, within 2 months after the first booster and within 3 months after the second booster dose. All participants completed a questionnaire. Participants who were SARS-CoV-2 RNA positive at any time were excluded from the study.

RESULTS

The median age of the participants was 41.1 ± 10.2 years (23-65) and 159 (74.7%) were females. The NAbs were detected in 72.2% and 49.7% of the participants and the median level was 57.7% and 19.1% at first and fourth month, respectively ($p<0.001$). The presence of chronic disease was an independent predictor for lower NAbs, while age, gender and immunosuppressive treatment had no effect. A strong positive correlation was found between anti-RBD IgG levels and NAbs in both measurements (rho: +0.70 $p<0.01$,+0.72 $p<0.0$, respectively).
Participants who received a single dose of BNT162b2 booster after two doses of Sinovac (n:59) had higher IgG levels and NAbs at the third and fourth measurements compared to a single dose of CoronaVac booster (n:11). Participants receiving two doses of BNT162b2 booster (n:22) had similar levels of anti-RBD IgG and NAbs in both measurements due to the rapid and high antibody response.

CONCLUSIONS

NAbs and IgG levels decreased significantly at 3 months after two doses of CoronaVac. Even a single mRNA booster increased the NAbs to >95%. Although there was a decrease in IgG levels in BNT162b2 booster recipients at 3 months, high levels of NAbs remained stable.

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ANTI-SPIKE/RBD IGG RESPONSE FOLLOWING COVID-19 VACCINATION WAS ASSESSED USING THE VIDAS® AUTOMATED ASSAY.

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BACKGROUND-AIM

The VIDAS® SARS-COV-2 IgG QUANT assay was used to evaluate the SARS-CoV-2 IgG antibody levels following vaccination with three different vaccines mostly used worldwide.

METHODS

VIDAS® SARS-COV-2 IgG QUANT is a quantitative immunoassay, based on the capture of specific antibodies using a recombinant SARS-CoV-2 receptor-binding domain (RBD) of the spike protein (RBD/S), using the Enzyme Linked Fluorescent Assay technique. Results are automatically reported in Binding Antibody Units (BAU)/mL. VIDAS® SARS-COV-2 IgG QUANT is metrologically traceable to the Working Standard WHO 21/234, which is calibrated to the International Standard WHO 20/136.
A total of 136 adult individuals without past medical history of SARS-CoV-2 infection, corresponding to 408 samples, vaccinated with Comirnaty® (Pfizer-BioNTech), Vaxzevria® (AstraZeneca) or Spikevax® (Moderna) following the recommended 2-dose vaccination protocol, were included. Among them, 129 individuals were aged from 18 to 65 years old. SARS-CoV anti-Spike/RBD IgG levels were assessed at three time points: before vaccination, before the second dose of vaccine, and 14-28 days after the second dose of vaccine.

RESULTS

For each timepoint and each vaccine, the number and proportion of individuals per interval of VIDAS® SARS-COV-2 IgG QUANT assay concentrations were established. After the first dose/before the second dose of vaccine, the proportion of individuals with an IgG concentration ε 25.0 BAU/mL (cut-off) was 96.0% (72/75) with Comirnaty®, 80.0% (16/20) with Spikevax® and 85.4% (35/41) with Vaxzevria®.
Following complete vaccination, all 136 adult individuals were detected positive. Seroconversion was therefore observed for all participants that were seronegative at baseline.

CONCLUSIONS

VIDAS® SARS-COV-2 IgG QUANT can quantitatively measure the levels of Spike-specific antibodies induced by COVID-19 vaccination, and their evolution.

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BIVALENT COVID-19 MRNA BOOSTER VACCINATION (BA.1 OR BA.4/BA.5) INCREASES NEUTRALIZATION OF MATCHED OMICRON VARIANTS

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BACKGROUND-AIM

The adoption of COVID-19 vaccines with the Omicron BA.1 or BA.5 variants has been pursued to improve protection against circulating Omicron variants. Early evidence indicated that this updated vaccine strategy may be effective, but it is still unclear whether a single booster dose with a bivalent vaccine would enhance neutralization of Omicron variants beyond that of original vaccines.

METHODS

In a prospective study, we investigated serum neutralization of SARS-CoV-2 variants at 0, 1 and 5 months after a bivalent BA.1 (n=12) or bivalent BA.4/BA.5 (n=22) booster of individuals who had previously received three vaccine doses of a monovalent vaccine. Additionally, we tested sera from vaccines with (n=31) or two booster doses (n=26) of the original monovalent mRNA vaccine as well as subjects after BA.1 (n=11), BA.2 (n=7) or BA.4/BA.5 (n=10) vaccine breakthrough infection (BTI). We performed live-virus neutralization tests (NT), using SARS-CoV-2 wildtype (D614G), Delta and Omicron variants (BA.1, BA.2, BA.5, XBB.1.5), and visualized neutralization data using antigenic cartography.

RESULTS

We found that a bivalent booster was more effective in inducing Omicron neutralizing activity. In particular, the bivalent-BA.4/BA.5 booster induced significantly higher neutralization titers to BA.5 than the 3- and 4-dose monovalent boosters. A comparison between sera obtained on the day of booster and those one month later showed that both the bivalent-BA.1 and –BA4/5 boosters yielded the strongest increase in the matched BA.1- and BA.5-titers, respectively. Similarly, BA.5-BTI after 2-4 monovalent vaccinations resulted in significantly higher BA.5- than BA.1-titers. The recent XBB.1.5 variant effectively evaded neutralizing activity elicited by current vaccines and/or previous variants.

CONCLUSIONS

Bivalent-BA.4/5 booster vaccination induced increased neutralization titers against the matched BA.5-variant compared with the monovalent boosters. This highlights the importance of vaccine matching to circulating variants.

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HUMORAL AND CELLULAR RESPONSES TO SARS-COV-2 OMICRON INFECTION IN VACCINATED AND ANTIGEN-NAÏVE INDIVIDUALS

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BACKGROUND-AIM

SARS-CoV-2 Omicron variants, which dominate the COVID-19 pandemic since early 2022, are B cell immune escape variants that elude the defence mechanisms induced by first-generation vaccines or infection with earlier SARS-CoV-2 variants. This study investigated the effect of Omicron infections on the humoral and cellular immunity in COVID-19-vaccinated vs. antigen-naïve individuals.

METHODS

Blood samples were collected from triple-vaccinated (ChAdOx1/BNT162b2/BNT162b2) individuals without (n=13) or with (n=13) Omicron breakthrough infection and from unvaccinated individuals after Omicron (n=15) or Wuhan infection (n=15). Anti-spike IgG was measured using the EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA (IgG) and Omicron ELISA (IgG), based on the S1 domain of the Wuhan and Omicron variant, respectively. IFN-© release was quantified using the EUROIMMUN Quan-T-Cell SARS-CoV-2 kit employing stimulation tubes with Wuhan or Omicron S1 antigen.

RESULTS

In vaccinees without SARS-CoV-2 infection, a significant decrease in anti-S1 IgG (minus 85.9%, P<0.0001) and IFN-© release (minus 73.3%, P=0.0062) was observed within 3.5 months following the third vaccine dose. Omicron breakthrough infections contracted 2 to 3.5 months after the third vaccination restored anti-spike IgG and IFN-© release to levels similar or above those measured 2 weeks post vaccination (P=0.0130 and P=0.4209). In antigen-naïve individuals, Omicron infection led to IFN-© results resembling those detected in the triple-vaccinated group 3.5 months after the third dose, whereas anti-S1 IgG levels were significantly lower than in vaccinees. Inter-ELISA and inter-IGRA comparison revealed very similar spike-specific results, irrespective of whether the tests were based on Wuhan or Omicron antigens.

CONCLUSIONS

There are substantial differences in immunity after Omicron infection in vaccinated and unvaccinated individuals. Considering the gradual decrease in immunity over time, unvaccinated individuals post Omicron infection are likely to have poor cross-protection against existing and possibly emerging SARS-CoV-2 variants. The detection of Omicron-induced immune responses in primed and antigen-naïve individuals supports the use of Omicron-adapted COVID-19 vaccines.

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I-MPOX: IMMUNE RESPONSE TO MONKEYPOX VIRUS. A 6-MONTH FOLLOW-UP IN NAïVE NEWLY VACCINATED HEALTHCARE WORKERS

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BACKGROUND-AIM

Monkeypox virus (MPXV) is closely related to Variola virus and vaccination with vaccinia virus (VACV) cross-protects, thus the new JYNNEOS vaccine was proposed to high-risk individuals. As comparative data on MPXV immune response in vaccinated subjects is still limited, we evaluated the immune response after two doses JYNNEOS administration and compared the data to VACV historically vaccinated subjects.

METHODS

Eight HCW received the JYNNEOS vaccine. Follow-ups: 1-month post-1st dose (October-November 2022), 1-month (November-December 2022) and 6-months (May-June 2023) post-2st dose. Twenty-three historically vaccinated HCW (VACV-HCW) and 19 naïve controls were included. VERO cells were used for isolation, titration and serological assays. An in-house immunofluorescence assay was set-up to evaluate the anti-MPXV IgM/IgG antibodies. Neutralizing antibodies (NT-Abs) against MPXV were defined by microneutralization. Peripheral blood mononuclear cells were isolated from heparinized whole blood to perform ELISpot assay using an in-house MPXV viral lysate.

RESULTS

In the JYNNEOS-HCW, the IgG titre significantly increases between first and second dose, while IgM and NT-Abs don't. There was a clear development of T-cell response in all but one JYNNEOS-HCW after 1-dose, but reached positive levels after two doses. In the VACV-HCW, 48% presented circulating IgG, but no IgM or NT-Abs, while T-cellular response was detectable in 60%.

CONCLUSIONS

In naïve subjects, a second dose boosts the serological response, while historically VACV-vaccinated controls retain a degree of protection even after years from vaccination. 1-month post-second dose, T-cell frequency is comparable between newly vaccinated and those having received VACV more than 40 years before.

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SPECIFIC IMMUNE RESPONSE AGAINST SARS-COV-2

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BACKGROUND-AIM

The SARS-CoV-2 coronavirus, causing COVID-19 disease, has killed almost 7 million people worldwide. For effective treatment and introduction of appropriate anti-pandemic measures, it is necessary to understand the development of postinfectious and postvaccination immune responses.

METHODS

In our study, we analysed anti-SARS-CoV-2 immune responses mainly against the spike (S) and nucleocapsid (N) proteins and also against membrane (M) or open reading frame (O) proteins. We isolated peripheral blood mononuclear cells and serum from convalescent COVID-19 patients. Sampling was done in two rounds one year apart. Most of the patients were vaccinated in between. SARS-CoV-2 seronegative donors served as controls. We used ELISpot assays to detect SARS-CoV-2-specific cells producing IFN-© (T cells) and IgG or IgA antibodies (B cells) using peptide mixtures (S, NMO) or recombinant proteins (S, N), respectively. We applied a CEF peptide mixture (consisting of selected epitopes from influenza A virus, cytomegalovirus, and Epstein-Barr virus) to assess the antiviral T-cell response and functionality of cells. SARS-CoV-2 specific antibodies were detected by ELISAs and a surrogate virus neutralisation assay.

RESULTS

Our results confirmed that SARS-CoV-2 infection induces: (1) memory B cells producing IgG targeted against S and N proteins and (2) T cells producing IFN-© after stimulation with S and NMO peptide mixtures. Interestingly, the T-cell response to the CEF mixture was lower in patients than in controls. We also detected that vaccination markedly enhanced levels of memory B cells (IgG) and T cells directed against S antigen. Comparison of B-cell responses showed a correlation between the number of IgG-producing B cells, and levels of circulating IgG and neutralizing antibodies.

CONCLUSIONS

Our study supports the importance of vaccination for immunity boosting and suggests the possibility of long-term disruption of the antiviral immune response caused by SARS-CoV-2.

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STABLE LEVELS OF UNRELATED TOXOID VACCINE RESPONSES AFTER COVID-19

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BACKGROUND-AIM

Lymphopenia is a common finding in COVID-19. This has raised concerns of COVID-19 affecting the immune system. Immune amnesia, where an infection can erase acquired immunity to other pathogens, is known to occur in measles infection. We hypothesized that a similar effect could be seen post-COVID-19. If there was a decline in humoral immunity, it would be evident in circulating antibody levels. In Finland, Diphtheria, Tetanus and Pertussis vaccines are administered as one injection and their coverage is good. We studied the effect of COVID-19 on humoral immunity through vaccine antibody levels.

METHODS

SARS-CoV-2 RT-PCR positive patients (n = 59) were recruited between March 2020 and February 2021 in Helsinki, Finland. Plasma samples were collected at 2-3 different occasions during and after infection. IgG antibodies to Diphtheria toxin, Tetanus toxoid and Borderella pertussis toxin were measured with a fully automatic EuroLabWorkstation ELISA (EUROIMMUN). Additionally, total IgG was determined using the clinically accredited Siemens Atellica CH IgG_2 assay.

RESULTS

We divided the samples into three timepoints by time since symptom onset (acute, δ35 d; convalescent 36-120 d; recovered, >120 d). We performed Friedman tests to test for differences in vaccine IgG and total IgG levels between the timepoints. No significant differences ($p < 0.05$) were found. We also performed paired Wilcoxon tests for only the hospital-treated patient population, where likewise there were no significant differences.
When comparing hospitalised and home-treated patients by timepoint (Mann-Whitney U test), there were no significant differences in vaccine responses. In total IgG, we found a significant difference ($p = 0.00322$) in the acute timepoint.

CONCLUSIONS

There is no humoral immunity boosting or decline during or after COVID-19 infection. In severe (hospital-treated) COVID-19, there appears to be a temporary decline in total IgG levels.

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THE IMMUNE RESPONSE AFTER SARS COV-2 INFECTION AND VACCINATION IN HIV-POSITIVE PATIENTS

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BACKGROUND-AIM

Clinical trials and real life studies have evaluated the efficacy and safety of vaccines for SARS-CoV-2 infection in diverse populations; nevertheless, there is still a paucity of data in immunocompromised patients, including those with HIV infection, who might develop persistent infections with high viral variability. The objective of this study was to assess the post-vaccination and post-infection humoral immune response in HIV-positive patients.

METHODS

Between April-August 2022, patients treated in an HIV regional center who agreed to participate in the study, were tested for anti-nucleocapsid IgG antibodies, anti-spike IgA, anti-Spike/RBD IgG. The anti-spike antibodies neutralizing capacity was assessed using a commercial surrogate virus neutralization test.

RESULTS

Out of the 104 enrolled patients (66.3% males, mean age - 38.43 years [18 – 76]). 93.3% were treated with cART for more than ten years; still, only 36.5% have undetectable HIV viral load at the time of the study. Anti NCP antibodies were present in 25% of the study patients, although only 10.6% of the patients acknowledged a prior SARS-CoV-2 infection, indicating a high percentage of asymptomatic infections. 38.5% were vaccinated either with a mRNA vaccine or a vector-based one, with the last dose received 6-12 months before enrollment. All vaccinated patients had detectable anti Spike antibody titers at 6-12 months, although most of them had not received a booster dose. The mean neutralizing capacity against the Omicron strain was 65%. Patients with hybrid immunity (infection and vaccination) had the highest anti-Spike IgG antibodies titers: mean BAU/ml 504.6 vs. 408.5 in those vaccinated only and 224.6 in those with prior infection, $p<0.0001$), the same trend was seen for anti-spike IgA, a surrogate for local immunity (mean reactivity 4.349 vs. 2.951 vs. 2.631, $p=0.0009$). Significantly lower anti S IgG and IgA antibody titers were present in patients with severe immunosuppression (CD4 count <200 cells/mmc).

CONCLUSIONS

SARS Cov-2 infection can remain asymptomatic even in HIV infected patients without viral suppression. HIV infected patients with severe immunosuppression have significant lower anti spike antibody titers and lower neutralizing capacity post infection and post vaccination.

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WHAT HAPPENS IN VACCINATED YOUNG PORTUGUESE WOMEN?

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BACKGROUND-AIM

To determine the distribution of HPV genotypes after vaccine introduction, ongoing evaluation of circulating HPV genotypes was conducted among young women already vaccinated.

METHODS

Young women immunized against HPV who had already initiated sexual activity are invited to participate in this evaluation and to complete a questionnaire about social, sexual and HPV vaccination data. Cervical samples are collected by Cytobrush and HPV genotyping were performed by the CLART®HPV2 (Genomic, Spain).

RESULTS

Between September 2014 and December 2022, 350 young women (mean age 22) have been evaluated; the mean age for 1st vaccination administration was 14.9 years, and for the first sexual intercourse was 16.9 years. HPV was detected in 34.9% (122/350) of the studied women; 74.6% (91/122) with high-risk genotypes, HPV 51 (15.2%) was the most frequent followed by HPV 58 with 6.1%. HPV 16 and HPV 18 were also detected in 3 women.
Low-risk HPV genotypes were detected in 29.5% (36/122) of the positive women, HPV 42 was the most frequent in 9.2% of the cases.
Vaccine data showed that 85.6% of vaccinated women in the immunization program had the sexual debut after vaccine administration, 54.9% of women vaccinated outside the immunization program were vaccinated after the sexual debut.

CONCLUSIONS

Portugal has a high HPV vaccination coverage. Our findings suggest the success of the HPV immunization program and reinforce the importance of vaccination before sexual debut. The results of this study are too early to affirm or to reject the hypothesis of HPV genotypes replace after introduction of the HPV vaccine.

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DATA ANALYSIS OF RUBELLA SEROPREVALENCE TREND FROM A SINGLE LAB IN SINGAPORE AND COMPARISON OF IN-HOUSE PLAGUE REDUCTION NEUTRALIZATION TEST WITH A COMMERCIAL IMMUNOASSAY IN DETERMINING IMMUNITY

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BACKGROUND-AIM

Rubella vaccination was first introduced in Singapore in 1976 and was included in the childhood immunization programme since 1990. A seroprevalence study in 1998 showed that the overall immunity in the population was 80.2%. In childbearing age (18–44 yrs), seropositivity was 84.2% in a 2004. The latest survey in 2018 showed that in children and adolescent seropositivity had reached 94.8%. Although there is a calibrated WHO international standard with a cutoff for immunity determined at 10 IU/mL, studies have shown that results from commercial assays correlated poorly.

METHODS

A single lab data of Rubella IgG results from 2007-22 (5 yrs intervals) from resident cohort were analysed. A selected number of sera with a spread of quantitative results by chemiluminescent microparticle immunoassay (Architect, Abbott) were selected and the PRNT was performed to compare results and to look at correlations in determining immunity status.

RESULTS

There is an increase in seropositivity trend over the years in the youngest age group (2-12 yrs old) reaching >90%. In the female reproductive age (21–45 yrs old), immunity increased with age. However, it was observed that within the same age group at the peak of reproductive age of between 21–35, seropositivity decreased over the 15 year period. The results from the samples were concordant by both methods.

CONCLUSIONS

High seropositivity in children demonstrates the effectiveness of the vaccination programme and its importance in eliminating Rubella. Interestingly, a decline of seropositivity is apparent in the 21-35 age group over the years. Although measurable antibodies by commercial assays are a proxy to demonstrate immunity, however, none-positivity does not equate to no protection in the immunocompetent vaccinated person. Trends should be monitored using a single commercial platform for comparability purposes. The results by CMIA were comparable to that of PRNT and could be used to correlate immunity to the commercial assay of choice. In countries with effective vaccination programmes, documented vaccination may be adequate without the need for testing due to variability of commercial assay results and high effectiveness of the vaccines.

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Neurovirology

DETECTION OF NEUROTROPHIC VIRUSES IN CEREBROSPINAL FLUID SAMPLES BY USING COMMERCIAL AND NON-COMMERCIAL ASSAYS

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BACKGROUND-AIM

Due to Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices which restricts of using laboratory developed tests (LDT) for a clinical diagnostics, we tested two commercial multiplex molecular biology assays AllplexTM Meningitis-V2, Seegene company (AM-V2) and Viral and Syphilis 16-well, AusDiagnostics company (VS-16w) to obtain more information about their quality for a possible replacement of our LDT.

METHODS

From June to October 2022, we prospectively tested 235 nucleic acid isolates (extracted by the QIAamp Viral RNA Mini kit, 140 ⌈l input, 100 ⌈l output) from fresh cerebrospinal fluid samples (CSF) by LDT and AM-V2. From January to February 2023 these frozen isolates were retested by VS-16w. The results from both assays were compared each other and with our LDT, which are regularly tested by QCMD international quality control.

RESULTS

Overall, 10 samples (4.3 %) were positive for enterovirus, 8 samples (3.4 %) for HHV7, 6 samples (2.6 %) for EBV, 4 samples (1.7 %) for VZV, 3 samples (1.3 %) for adenovirus and HHV6, 2 samples (0.9 %) for HSV and parvovirus B19, 1 sample (0.4 %) for CMV and none for parechovirus, mumps virus, BK virus, JC virus and monkeypox virus using all commercial and non-commercial tests. Diagnostics of enteroviruses was better by using both commercial tests than LDT. Enterovirus sensitivity of AM-V2 was lower compared with VS-16w (66,7 %), specificity was 99,6 %. The sensitivity of VS-16w was lower for HSV (50 %), VZV (75 %), HHV6 (66.7 %), EBV (33.3%) and CMV (0%) than LDT. On the other hand, the sensitivity for HHV7 (25 %) and EBV (33.3 %) was lower for LDT than VS-16w.

CONCLUSIONS

The European regulation of in vitro diagnostics will significantly impact the microbial laboratory results in the future. It is necessary to compare commercial tests with LDT to ensure that they are of good quality. Based on the results of our study, we could improve diagnostics of enteroviruses by introducing of commercial tests. Conversely, sensitivity for other viruses such as HSV and VZV will be decreased. Using a more concentrated nucleic acid could catch up with the required PCR sensitivity. Supported by the project for conceptual development of research organization 00064203

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Neurovirology

EVALUATION OF BIO-SPEEDY MENINGITIS/ENCEPHALITIS PANEL FOR THE ETIOLOGICAL DIAGNOSIS OF CENTRAL NERVOUS SYSTEM INFECTIONS: A SINGLE-CENTER STUDY.

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BACKGROUND-AIM

The Bio-Speedy Meningitis/Encephalitis (ME) (Bioeksen, Istanbul, Turkey) panel is a multiplex PCR test aiming for the simultaneous, rapid (~1-h) detection of 17 pathogens (E. coli K1, H. influenzae, L. monocytogenes, N. meningitidis, S. pneumoniae, S. agalactiae, CMV, Enterovirus (EV), HSV-1 and 2, HHV-6, HPeV, VZV and Cryptococcus gatti/neoformans) from cerebrospinal fluid (CSF) specimens for in vitro diagnosing of CNS infections.
This study evaluated the Bio-Speedy ME panel's performance in a single center.

METHODS

This study included CSF specimens of 88 patients with suspected CNS infection between June 2022 and March 2023. The results of the Bio-Speedy panel were compared with gram staining/bacterial culture and the FTD Viral meningitis Kit (Fast Track Diagnostics Ltd, Luxemburg) (CE-marked for IVD use in the EU) (HSV-1, HSV-2, VZV, EV, Mumps virus, HPeV). Nucleic acid purification of viruses was performed with EZ1&2 Virus Mini Kit (Qiagen, Germany) in the EZ1 system.
Test results supported by clinical/radiologic findings and CSF cell count [UF-5000 flow cytometry system (Sysmex, Japan)] were interpreted as true positive (TP), and inconsistent results were interpreted as indeterminate positives in terms of clinical diagnosis (IP).

RESULTS

At least one pathogen was detected by at least one method in 16 (18.2%) samples, of which 12 (13.6%) were considered TP.
Nine of them [VZV (n=3), EV (n=1), S.pneumoniae (n=2), N. meningitides (n=1), H. influenzae (n=1)] were detected by Biospeedy panel, four of them were detected by FTD and also BioSpeedy panels [VZV (n=3), EV (n=1)]. One bacteria grew only culture (S.pyogenes), and two viruses detected only by FTD panel (HSV-2 and Mumps virus).
A total of five pathogens [S.pneumoniae (n=2), HHV-6 (n=2), HHV-7 (n=1)] detected only by Biospeedy were considered IP.

CONCLUSIONS

The Biospeedy molecular diagnostic panel includes a wide range of possible pathogens on a broad scale to avoid false negative results and ensure an accurate diagnosis. However, test results should interpret or confirm according to clinical and laboratory examination -especially bacteria- to avoid method-induced IP results and unnecessary treatment applications.

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Neurovirology

HOST GENETIC VARIANTS INFLUENCING NK CELL ACTIVITY IN PATIENTS WITH LONG COVID AND CHRONIC FATIGUE SYNDROME

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BACKGROUND-AIM

Natural killer (NK) cell dysfunctions such as impaired NK cell activation and cytotoxicity have been described in post-viral fatigue syndromes. The aim of the current study was to identify whether there are yet unknown associations of Long COVID fatigue syndromes as well as of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) with host genetic variants influencing the NK cell response and to identify risk factors predisposing for the development of these fatigue syndromes.

METHODS

We included 30 well-characterized Long COVID patients with persistent fatigue, post-exertional malaise, autonomic dysfunction and/or orthostatic intolerance, and 30 age- and sex-matched SARS-CoV-2 convalescent individuals without any Long COVID symptoms. Additionally, we included a cohort of 39 well-characterized ME/CFS patients. All were tested for the CD16A (Fc©IIIa) V/F dimorphism, influencing the NK cell-mediated ADCC response, HLA-E*0101/*0103 and NKG2C wt/del variants, affecting specific NK cell activation, and the rs9916629 C/T polymorphism, influencing NK cell subpopulations and functions. Furthermore, HCMV serostatus was assessed in a subset of participants. All patient cohorts as well as healthy SARS-CoV-2 convalescent individuals were further compared to over 400 healthy control persons serving as population control.

RESULTS

Mean age of all participant cohorts ranged between 34 and 40 years, and between 75% and 80% of individuals per cohort were female. Statistically significant differences were observed in the distribution of the NKG2C deletion markers especially between Long COVID patients as well as healthy SARS-CoV-2 convalescent patients and the overall population. Also, combinations of NKG2C with either rs9916629 or HLA-E variants were unevenly distributed between these groups. No differences were observed between ME/CFS patients and the overall population. Prior HCMV infection, which is known to shape the NK cell response, was not differently distributed between any patients and controls.

CONCLUSIONS

There is some evidence that genetic variations in NK cell influencing markers are associated with the development of Long COVID post-viral fatigue syndromes. However, the data assessed in the current investigation require confirmation and clarification by further and larger studies.

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Neurovirology

NEUROTROPIC VIRAL INFECTIONS OF THE CENTRAL NERVOUS SYSTEM: A RETROSPECTIVE ANALYSIS (2018-2022)

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BACKGROUND-AIM

To perform a retrospective evaluation of the impact of neurotropic virus in central nervous system (CNS) pathologies, according to laboratory results, and epidemiological/clinical data.

METHODS

1665 cerebrospinal fluid (CSF) samples, collected from 01/2018 to 12/2022, from patients suspected of CNS infection were sent to the Portuguese NIH for neurotropic virus determination. A commercial multiplex real-time PCR kit for EBV, HCMV, HAdV, HSV-1, HSV-2, VZV, EV, HpeV, parvovirus B19, HHV-6, and HHV-7 identification was used.

RESULTS

290/1665 (17.4%) CSF samples provided a positive result: 112 (38.6%) EBV+, 45 (15.6%) HHV-7+, 26 (9.0%) VZV+, 23 (8.0%) EV+, 17 (5.9%) HSV-1+, 16 (5.5%) HHV-6+, 15 (5.2%) CMV+, 10 (3.5%) HSV-2+, 8 (2.8%) HAdV+, 2 (0.7%) HPeV+ and 2 (0.7%) parvovirus B19+. Coinfection of EBV with other viruses was observed in 14 (4.8%) cases.
With the exception of two children aged 2 years old, 72/290 (24.8%) positive cases were determined in immunocompromised patients aged ε19 years; 218/290 (75.2%) were immunocompetent with ages from one month to 90 years old.
The clinical history underlying positive cases were encephalitis (116/290) with intrinsic signs/symptoms of altered mental status, fever, headaches, and seizures; meningitis (68/290) associated with fever, headache, vomiting, and neck stiffness; meningoencephalitis (28/290) presenting fever, headache, and aphasia; abnormal brain imaging (18/290); and Dementia Syndrome (12/290). The remaining 48 positive cases evidenced paresis, myelitis, neuritis, ataxia, and Guillain-Barre Syndrome.

CONCLUSIONS

We believe that the most common neurotropic virus, HSV-1, HSV-2, and VZV cases were diagnosed at the hospital, and for that reason, they were less detected in our sampling; in fact, the Portuguese NIH is the National Reference Laboratory and acts as a second response. However, this is a scenario requiring further evaluation.
EBV and HHV-7's frequency may indicate an underestimated pathogenic role in CNS disease, which should alert clinicians to consider them upon CNS infection suspicion.
The multiplex PCR approach for neurotropic viruses constitutes a valuable tool for clinical diagnosis and disease monitoring, enabling the detection of neglected viruses, while reducing cost, labor, execution time, and CSF consumption.

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Neurovirology

PARECHOVIRUS-A FINDINGS IN FINLAND DURING YEARS 2012-2019

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BACKGROUND-AIM

The Parechovirus-A (PeV-A, formerly Human parechovirus, HPeV) species comprises 19 types. These are common around the globe, with PeV-A1 being the most common type. PeV-As infect mostly children and cause mild gastroenteritis and respiratory infections. However, especially type 3 can cause serious sepsis like disease and central nervous system infections, such as meningitis and encephalitis.

METHODS

Helsinki University Hospital laboratory (HUSLAB) started PeV-A diagnostics in 2012 as a pilot study from serum and cerebrospinal fluid samples (CSF) followed by implementing the molecular method into daily diagnostics.

RESULTS

During 2012 seven PeV-A caused sepsis-like disease cases were detected. After several low prevalence years, with only up to one case annually, an outbreak was detected in 2018 with nine hospitalized patients. The outbreak appeared in August and peaked in September/October. All PeV-A positive patients were under three months-old, and two of them needed treatment at the intensive care unit (ICU). PeV-A3 was typed from a 1-week-old patient treated in ICU, however, from the other PeV-A positive ICU patient no sample was available for typing. Typical symptoms were fever (>38°C), irritability, tachycardia, marmorizing skin and rash. In year 2019, only one PeV-A was detected from a 2.5-month-old girl from a CSF sample.

CONCLUSIONS

Most of the found PeV-As have been typed as PeV-A3, but interestingly the outbreak of year 2012 was caused by a rare PeV-A4 strain. Furthermore, one PeV-A5 was detected during the 2018 outbreak.

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Neurovirology

VIRAL CENTRAL NERVOUS SYSTEM INFECTIONS: 10 YEARS OF DIAGNOSTIC EXPERTISE

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BACKGROUND-AIM

We evaluated Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Varicella Zoster Virus (VZV), Enterovirus (EV), Cytomegalovirus (CMV) and Human Herpesvirus 6 (HHV-6) in cerebrospinal fluid (CSF) from patients with clinical suspicion of acute central nervous system (CNS) infection.

METHODS

Results obtained on CSF samples over the past 10 years at Microbiology Unit of Sant'Orsola Polyclinic were retrospectively studied. From 2013 to 2017, extraction was performed with the QIAsymphony SP instrument (Qiagen, Germany) and the ELITe MGB® kits (ELITechGroup, Italy) were used for real-time PCR amplification. From 2018, the ELITeInGenius SP 200 and ELITe MGB® kits (ELITechGroup, Italy) were used on the ELITeInGenius® instrument.

RESULTS

From January 2013 to December 2022, virological examinations were performed on 4026 CSF and a positive result was observed in 291 samples (7%). In particular, 81 (28%) samples were positive for VZV, 63 (22%) for EV, 58 (20%) for HHV-6, 47 (16%) for HSV-1, 24 (8%) for CMV and 18 (6%) for HSV-2. No cases of EV meningitis were detected in the years 2020-2021. Focusing our attention on the 58 patients with HHV-6 positivity, the determination of blood and hair follicle viral load permitted the identification of 26 cases of HHV-6 integration, 26 of HHV-6 latency, and only in 6 pediatric cases an acute HHV-6 infection associated with CNS involvement. Considering the patient's immune status, in immunocompetent CNS infections were mostly caused by VZV (35%), followed by EV (31%), while in immunocompromised were prevalently caused by CMV (63%). In relation to the patient's age, it was observed that CNS infections in pediatric patients were mainly caused by EV (55%) and VZV (6%), while in adult patients VZV was prevalent (38%), followed by EV (22%).

CONCLUSIONS

Viral CNS infections are related to the patient's age and immunological status. A positive HHV-6 result on CSF has to be interpreted with caution and is not by itself exhaustive for patients' final diagnosis, always requiring further diagnostic investigation on blood and eventually on hair follicle.
The absence of EV meningitis during the years 2020-2021 are probably due to the hygiene measures implemented to prevent SARS-CoV-2 infection.

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One Health

BHSCT ICT UPLOAD TEST

B. Test [1]
[1]*BHSCT*

BACKGROUND-AIM

Video provides a powerful way to help you prove your point. When you click Online Video, you can paste in the embed code for the video you want to add. You can also type a keyword to search online for the video that best fits your document.
To make your document look professionally produced, Word provides header, footer, cover page, and text box designs that complement each other. For example, you can add a matching cover page, header, and sidebar. Click Insert and then choose the elements you want from the different galleries.
Themes and styles also help keep your document coordinated. When you click Design and choose a new Theme, the pictures, charts, and SmartArt graphics change to match your new theme. When you apply styles, your headings change to match the new theme.
Save time in Word with new buttons that show up where you need them. To change the way a picture fits in your document, click it and a button for layout options appears next to it. When you work on a table, click where you want to add a row or a column, and then click the plus sign.
Reading is easier, too, in the new Reading view. You can collapse parts of the document and focus on the text you want. If you need to stop reading before you reach the end, Word remembers where you left off - even on another device.

METHODS

Video provides a powerful way to help you prove your point. When you click Online Video, you can paste in the embed code for the video you want to add. You can also type a keyword to search online for the video that best fits your document.

RESULTS

Some great results!

CONCLUSIONS

Conclusion

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One Health

CHRONOLOGICAL MONITORING OF BAT CORONAVIRUSES AND ANALYSES ON THEIR RELATIONSHIP WITH BAT ECOLOGY

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BACKGROUND-AIM

Several natural bat habitats were selected and chronologically monitored for the bat coronaviruses with ecological data of the habituated bats to study their relationship and potential risk variables for bat coronavirus circulations in the bat population.

METHODS

Between January 2021 and December 2022, a total of 1,531 samples were collected, including oral swabs, guano, urine samples from 15 species of bats in their natural habitats along with their ecological data such as sex, age, body weight, forearm length, and collection data/locations. The sampling was performed under the approval from National institute of Ecology in Korea. The collected samples were tested for coronaviruses using consensus-primer based RT-PCR as well as NGS analysis from the selected pooled samples. The relationship between coronavirus positives and bat ecological data were further analyzed with phylogenetic tree and logistic regression analysis.

RESULTS

Among the tested samples, alphacoronaviruses were detected in 4.89% (75/1,531) of the samples from seven bat species, M. hilgendorfi (21.05%; 4/19), M. petax (19.23%; 5/26), M. macrodactylus (12.50%; 26/208), M. aurascens (5.66%; 3/53), R. ferrumequinum (3.76%; 21/559), M. fuliginosus (3.11%; 12/386), and M. bombinus (2.11%; 4/190). Based on the partial RDRP sequences-based phylogenetic analysis, there were around six clades of alphaCoV circulating in those bat species. Although some of them were grouped by either region- or bat species-specific subclades, alphaCoVs in this study seemed related with genus of bat species. The body condition index (BCI) was also compared between alphaCoV positive and negative bats, showing that regional- and bat species-specific differences of mean BCIs between them. For example, reduced mean BCI was observed in the alphaCoV-positive bats of M. auracens and M. macrodactylus. In the logistic regression analysis, sample type, age of bats, and collection date were significantly correlated with alphaCoV positive in the RT-PCR.

CONCLUSIONS

Bats as the natural hosts of diverse coronaviruses, we could observe that alphaCoVs were endemically circulating in the bat population, sometimes their infection was associated with the reduced BCI in some bat species, which implied that there might be seasonal pattern of alphaCoV circulation in bat population.

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One Health

INDOOR AIR SAMPLING IS A SENSITIVE INDICATOR FOR THE PRESENCE OF INDIVIDUALS INFECTED WITH RESPIRATORY PATHOGENS

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BACKGROUND-AIM

By allowing non-invasive detection of respiratory pathogens in community settings, indoor air sampling has shown promise as a novel method for disease surveillance and assessment of airborne transmission risk. However, it is unclear whether the detection of airborne pathogens corresponds with the presence of infected individuals and their pathogen shedding rate. In this study, we aimed to correlate the presence and concentration of respiratory pathogens in air samples and used paper tissues (UPT) of individual persons in the same room.

METHODS

This observational study was conducted in a nursery unit. On one day per week for 10 consecutive weeks, we sampled the air (AerosolSense, Thermo Fisher Scientific) for 2 hours and collected UPTs of 21 participating children and child carers for 4 hours. We did not actively take samples from participants, but collected all paper tissues that had been used to wipe their noses. For each sampling day, UPTs were pooled into one sample per individual participant. All samples were tested for 29 respiratory pathogens using multiplex qPCR.

RESULTS

The mean number of persons present during sampling was 22.6. On each sampling day, a mean of 7.4 attendees used a paper tissue which was collected. In these UPTs, 2.3 different pathogens were found per sample and 5.6 per sampling day. The pathogens most often detected in UPT were human enterovirus, including rhinovirus (9), Streptococcus pneumoniae (9), human cytomegalovirus (8) and human bocavirus (7). The overall sensitivity of air sampling for detecting the pathogens present in UPT was 84% (95% CI 71-93%). Human coronavirus NL63 was detected in UPT three times but never in the air sample. However, Pneumocystis jirovecii and herpes simplex virus type 1 were regularly detected only in air samples. The PCR cycle threshold value was significantly higher in air samples than in corresponding UPT samples for human enterovirus (+6.2 cycles), human coronavirus HKU-1 (+5.3 cycles), Streptococcus pneumoniae (+4.8 cycles), and human adenovirus (+1.9 cycles).

CONCLUSIONS

Our results indicate that air sampling is a sensitive indicator for the presence of individuals infected with respiratory pathogens. It should be investigated further as a novel sampling strategy for disease surveillance and assessment of transmission risk.

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One Health

INSAFLU-TELEVIR: AN OPEN WEB-BASED BIOINFORMATICS SUITE FOR VIRUS METAGENOMIC DETECTION AND ROUTINE GENOMIC SURVEILLANCE

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BACKGROUND-AIM

Implementation of virus metagenomics diagnostics and routine genomic surveillance can be particularly challenging due to the lack of bioinformatics tools and/or expertise. In order to face this challenge, we have previously developed INSaFLU (https://insaflu.insa.pt/), a free bioinformatics platform for virus NGS data analysis. Here, we expanded its genomic surveillance component, and developed a brand new module (TELEVIR) for metagenomics virus identification.

METHODS

INSaFLU-TELEVIR is compatible with Illumina, Ion Torrent and Oxford Nanopore technologies (ONT) data and is freely available at https://insaflu.insa.pt/ (online tool), https://github.com/INSaFLU (code, including Docker installation https://github.com/INSaFLU/docker) and https://insaflu.readthedocs.io/en/latest/ (tutorial/documentation). It is mainly written in Python and is available through a Django web framework.

RESULTS

In this study, INSaFLU (https://insaflu.insa.pt/) platform was strengthened with new surveillance functionalities, such as: i) SARS-CoV-2 lineage classification; ii) Nextclade analysis; iii) Nextstrain phylogeographic analysis (SARS-CoV-2, seasonal and avian influenza, mpox, RSV, as well as a “generic” build for other viruses); or, iv) screening for mutations of interest (https://github.com/insapathogenomics/algn2pheno). In parallel, a new module (TELEVIR) for virus detection was developed (https://insaflu.readthedocs.io/en/latest/metagenomics_virus_detection.html). TELEVIR allows running complex workflows, covering several combinations of steps (e.g., with/without Viral enrichment/Host depletion), classification software (e.g., Kaiju, Kraken2, Centrifuge, FastViromeExplorer) and databases (RefSeq viral genome, Virosaurus, etc), while culminating in user- and diagnosis-oriented reports. Ultimately, to potentiate real-time virus detection during MinION runs, we developed findONTime (https://github.com/INSaFLU/findONTime), a tool aiming at reducing costs and the time between sample reception and diagnosis.

CONCLUSIONS

INSaFLU-TELEVIR versatility and functionality is expected to supply public health laboratories and researchers with a user-oriented bioinformatics framework that can potentiate a strengthened and timely metagenomics virus detection and routine genomics surveillance.

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One Health

INVESTIGATING THE USE OF THE TWIST COMPREHENSIVE VIRAL RESEARCH PANEL AS A TOOL FOR VIRAL METAGENOMICS

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BACKGROUND-AIM

Molecular methods are commonly used for viral diagnosis but there are limitations to this approach. These include the number of targets that be detected in one diagnostic panel and the ability of such panels to detect new and emerging pathogens. In contrast, a metagenomics approach to viral diagnostics increases the scope for pathogen detection, and, the recent SARS-CoV2 pandemic has highlighted the need for more adaptable diagnostic tools. We evaluated the performance of a commercial next-generation sequencing (NGS) assay, the Comprehensive Viral Research Panel (Twist Biosciences), designed to detect new and existing viral agents on a viral metagenomics EQA panel.

METHODS

The Twist Comprehensive Viral Research Panel was used to characterize the QCMD 2021 Viral Metagenomics NGS EQA Pilot Study (NGSmeta_21)) according to the manufacturer's instructions. Twist synthetic EVD68 viral control (spiked into a background of human reference RNA (Agilent)) was used as a positive control. A negative control consisting solely of human reference RNA was processed in parallel. Enriched libraries were sequenced with 2x75bp paired-end reads on the Illumina MiSeq platform and the output was analysed using the One Codex platform.

RESULTS

The NGSmeta_21 panel consisted of five samples; one negative and four positive. In the former the Twist CVR Panel detected no viral reads. The four positive EQA samples contained varying concentrations (Log^{10} Copies/mL) of Enterovirus D68, Herpes simplex virus 1 and/or 2 and Influenza A (H3N2 or H1N1). The Twist CVR panel correctly identified the organisms in each of the four specimens. In addition to the expected pathogens, the Twist CVR panel identified a high proportion (26.1%) of Hepacivirus C virus reads in one of the EQA samples. Whilst the viral load was seen to influence the abundance of reads in the output, there appear to be other factors which introduce bias in the results. These may be the genome composition or the viral species contained in each of the EQA samples.

CONCLUSIONS

There was excellent concordance with the expected results of the EQA panel and the output from the Twist assay. Testing of patient samples will provide further evidence of the potential utility of this approach to viral diagnostics.

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Respiratory viruses

AMINO ACID VARIABILITY OF SMALL-MOLECULE INHIBITORS AND MABS TARGET SITES IN GLOBALLY CIRCULATING RSV STRAINS

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BACKGROUND-AIM

To characterize the extent of amino acid (aa) variability in target sites of small molecule inhibitors (SMIs) and monoclonal antibodies (mAbs) against RSV by analyzing globally circulating RSV deposited sequences.

METHODS

1367 full-length RSV sequences from drug-naïve patients were downloaded from NCBI database (one sequence/patient). RSV type was inferred by Maximum likelihood phylogeny. Genetic distance (Maximum Composite models) and aa variability (Entropy) were evaluated in N, F, G, and L proteins (SMIs and mAbs targets) against RSV type, while selective pressure (dN/dS) by Fubar and MEME tools, retaining only positions under positive selection with both methods.

RESULTS

Retrieved sequences included 845 (61.8%) type A and 522 (38.2%) type B RSV, mostly (1214, 88.8%) isolated before 2020. Sequences mainly came from Europe (47.5%) and North- and South-America (33.9%).
The genetic diversity was the lowest in N protein, followed by F, G and L in both RSV types. No N aa positions were under positive selection, and only 6 N aa positions (localized in N-ntd and N-ctd domains) showed different entropy between A and B types (delta-H>0.1, $p<0.05$). Differently, 26 F (8 localized in antigenic sites Ø,I,II and V) and 105 G aa positions were characterized by different entropy in A and B types (delta-H>0.1, $p<0.05$), confirming divergent evolutionary pathways of G protein between RSV types.
Finally, L protein was characterized by 76 aa positions (32 localized in RdRp) showing different entropy between A and B types (delta-H >0.1, $p<0.05$) and no aa positions under positive selection.
None of the positions characterized by different entropy and selective pressure were at drug-resistant sites. Only 32 (3.3%) sequences carried aa mutations conferring resistance to at least one mAb or SMI. One B strain carried a combination of F mutations (K272N+F488L) conferring full-resistance to Pavilizumab and SMIs against F, respectively.

CONCLUSIONS

RSV strains circulating worldwide showed a lower degree of genetic variability in N protein compared to the other drug targets and little residue variability at positions associated to drug-resistance.
Continuous efforts in monitoring RSV genomic evolution, especially at mAbs and SMIs targets, are needed to guide future therapeutic and preventive strategies development.

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Respiratory viruses

ASSESSMENT OF THE SARS-COV-2 TESTING PROCESSES USED IN THE DIAGNOSIS AND MEDICAL MANAGEMENT OF HOSPITALISED COVID19 PATIENTS IN NHS SCOTLAND.

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[1]*NHS Lothian*

BACKGROUND-AIM

Studies of SARS-COV2 genetics during chronic infection have reported the emergence of antigenically diverse variants. In NHS Scotland a laboratory service has been set up to perform whole genome sequencing (WGS) on patients at risk of chronic infection, and all hospitalised patients. Sequencing of SARS-COV2 in these patient populations may allow for improved infection control practices and a deeper understanding of SARS-COV2 evolution.
The aim of this study was to assess if SARS-COV2 positive PCR samples were obtained from these patients and referred for WGS.

METHODS

A list of patients that received Ronapreve, Remdesivir or Paxlovid between December 2021 and August 2022 was obtained. A review of patient records was performed to identify the microbiological test used to diagnose SARS-COV2 infection, whether patients received follow-up PCR tests, and the number of PCR positive samples that underwent WGS. Length of inpatient stay and COVID19 vaccination status were also noted.

RESULTS

Prior to treatment, a positive laboratory PCR test was documented for 72% of the 43 Ronapreve-treated and 95% of the 188 Remdesivir-treated patients. Patients receiving these two treatments were managed in hospital, and all positive PCR tests were referred for WGS. In contrast, the 310 patients treated with Paxlovid were managed in the community via NHS Lothian's outpatient antimicrobial service. A positive laboratory PCR test was documented in only 118 (38%) of these patients. There was evidence of point of care testing in this group, but without laboratory PCR confirmation referral for WGS was not possible. Analysis of WGS data from treated patients did not flag any novel SARS-COV2 variants.

CONCLUSIONS

This study showed the importance of understanding SARS-COV2 testing pathways for patients with COVID19 to improve the flow of priority samples into the laboratory for PCR and WGS analysis. Future work will focus on community pathways to ensure clear messaging on the need to obtain a laboratory PCR result, which can be referred for WGS. The process for obtaining swabs from patients receiving treatment for COVID19 could be optimised to include a PCR swab for epidemiological surveillance. Ongoing analysis of WGS data from patients with sequential PCR positive samples will assess genetic diversity during infection.

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Respiratory viruses

CHANGES IN RESPIRATORY VIRUS DYNAMICS FOLLOWING THE RELAXATION OF NON-PHARMACEUTICAL INTERVENTIONS IN SOUTH KOREA

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BACKGROUND-AIM

The coronavirus disease (COVID-19) pandemic draws to a close, and South Korea has relaxed various non-pharmaceutical interventions (NPIs) implemented to suppress the infection spread. This study aimed to investigate the changes in the dynamics of respiratory virus infections following this relaxation.

METHODS

Polymerase chain reaction (PCR) results pertaining to respiratory viruses from a tertiary care hospital in South Korea, collected over a period of 7 years (April 2016−March 2023), were analyzed. The data were obtained using the respiratory virus panel (Anyplex II RV 16 or Allplex Respiratory Panels, Seegene, Seoul, South Korea).

RESULTS

The number of respiratory virus infections gradually increased with the relaxation of NPIs. Before the pandemic, human parainfluenza virus (HPIV) outbreaks occurred in late spring to summer; HPIV3 was the dominant strain. In 2020, the number of HPIV-infected patients was extremely low. In 2021, HPIV3 spread occurred between late autumn and early winter, which was 5−6 months later than usual. In 2022, HPIV3 did not show a major outbreak; however, there were signs of an epidemic starting from early spring 2023, indicating a return to the seasonality in the pre-COVID-19 outbreak period. Between 2016 and 2019, respiratory syncytial virus (RSV) exhibited a seasonal pattern from late fall to winter. In 2020−2021, there were almost no RSV infections; however, from the end of 2021, RSV B spread occurred, and this outbreak was approximately 2 months later than usual. In 2022, there was an RSV outbreak between late autumn and winter, similar to that during the pre-COVID-19 period. Human metapneumovirus outbreaks are common in spring; However, there were no infections in the spring of 2020 and 2021. In 2022, an outbreak occurred in the fall, which was approximately 6 months later than usual. Human bocavirus showed its usual seasonality from 2021, excluding 2020. Human rhinovirus exhibited a pattern of outbreaks in spring and autumn, including during the COVID-19 period.

CONCLUSIONS

The relaxation in NPIs has resulted in a gradual increase in respiratory virus infections, initially displaying a seasonality different from that during the pre-COVID-19 period but eventually returning to their original seasonality.

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Respiratory viruses

CIRCULATION OF SARS-COV-2 VARIANTS IN HOSPITALIZED AND NON-HOSPITALIZED PATIENTS FROM 2021 TO 2022: A LARGE SINGLE-CENTRE STUDY

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BACKGROUND-AIM

SARS-CoV-2 variants have been detected since its initial emergence including Alpha, Beta, Gamma, Delta and Omicron. The aim of this study was to evaluate the circulation of viral variants among non-hospitalized (group A) and hospitalized (group B) patients in Milan/Lombardy area, Northern Italy, using a combined approach for variant identification: Real-Time Polymerase Chain Reaction (RT-PCR) and Whole Genome Sequencing (WGS)

METHODS

SARS-CoV-2 RNA was detected using RT-PCR multi-target assays: Allplex™ SARS-CoV-2 (Seegene, South Korea), SARS-CoV-2 RT-qPCR (PerkinElmer, USA), SARS-CoV-2 ELITe MGB kit (ELITechGroup, France) and Xpert Xpress SARS-CoV-2 (Cepheid, USA) assays. SARS-CoV-2 positive samples with Ct<28 have been selected for genotyping (criteria: random sampling, arrival from area at risk of variants, cluster of outbreaks, potential reinfection, vaccinated subjects). The analysis was performed on respiratory samples collected from 23 January 2021 (week 1) to 3 August 2022 (week 80). Variants identification was performed both through a specific RT-PCR commercial assay, the Allplex SARS-CoV-2 Variants I + II kit (Seegene), and WGS on iSeq100 platform (Illumina, USA), using CleanPlex SARS-CoV-2 Panel kit (Paragon Genomics, USA)

RESULTS

A total of 5198 SARS-CoV-2 positive samples were tested for variant identification (4242 samples from group A and 956 from group B). In groups A and B, we detected: B.1.177 (5% vs 14%), Alpha (24% vs 29%), Beta/Gamma (1% vs 1%), Delta/Delta plus (49% vs 18%), Omicron (20% vs 38%), other lineages including B.1.525, B.1.1.318, C.11, C.36 (2% vs 1%). In group A Alpha variant replaced B.1.177 strain faster than group B: a weekly positivity rate ε75% was reached at week 4 vs week 8, respectively, while Delta emerged 8 weeks earlier in group A than in group B. Similarly, Omicron was detected for the first time in group A 5 weeks earlier than in B (week 43 vs 48) but its sub-variants co-circulation started from week 54 (BA.2) and week 70-71 (BA.4/BA.5) in both groups

CONCLUSIONS

The combination of RT-PCR and WGS represented a cost-effective approach for a high-throughput variant surveillance allowing the detection of new variants. In addition, the most relevant variants (Alpha, Delta and Omicron) emerged earlier in non-hospitalized vs hospitalized patients

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Respiratory viruses

COMPARATIVE STUDY FOR PERFORMANCE EVALUATION OF TWO CE-IVD TESTS FOR DETECTION OF ADENOVIRUS, PARAINFLUENZAVIRUS TYPE 1-4, HUMAN METAPNEUMOVIRUS RHINOVIRUS AND ENTEROVIRUS

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BACKGROUND-AIM

Nucleic acid amplification techniques (NAAT) are now the gold standard for identification of the viral respiratory tract infections due to their higher sensitivity and specificity when compared to traditional techniques. Multiplex formats solve the practical shortcoming of detecting only the infectious agent that is searched for. Here the evaluation of the TaqPathTM Respiratory Viral Select Panel (TP) for the detection of adenovirus (ADE), parainfluenza virus (PIV) type 1-4, human metapneumovirus (hMPV), rhinovirus and enterovirus (HRV/EV) against the RespiFinder 2Smart (R2S) is presented.

METHODS

311 archived nasopharyngeal swabs (NPS), collected in the period from 2007-2019 in Europe previously found to be positive (GRACE and PREPARE project) for the presence of ADE, PIV 1-4, hMPV, HRV/EV and 100 negative archived NPS were tested in parallel with the TP assay (Thermo Fisher Scientific) and the R2S test (PathoFinder). FTD Respiratory Pathogens 21 assay (FTD) (Siemens Healthineers) was used to resolve discrepant results. All tests were performed according to their instructions for use. 5 samples were excluded from analysis due to invalid results or lack of sample volume. An expanded gold standard was used to calculate clinical sensitivity and specificity of both assays.

RESULTS

Positive and negative percent agreement varied between 66.0%-100%, and between 94.5%-98.4%, respectively. Seventy-four samples were resolved by the FTD assay: the majority of discrepant results were noticed for PIV1-4 (positive by TP, negative by R2S) and for HRV (negative by TP, positive by R2S). After discordant resolution, sensitivity of the TP assay for the detection of ADE, PIV1-4, hMPV and HRV/EV was 100%, 100%, 97.9% and 69.3%, while specificity was 99.5%, 99.7%, 99.7% and 99.3%, respectively. Sensitivity and specificity of the R2S were 96.0%, 91.6%, 87.1% and 76.6% and 99.0%, 100%, 100% and 99.7% for the detection of HRV/EV, hMPV, ADE and PIV1-4.

CONCLUSIONS

The TP assay has a very high specificity for all targets (>99%), and a very high sensitivity for ADE, PIV1-4 and hMPV (>97.8%). As the test is not designed for detection of all Rhinovirus C species, the sensitivity observed for Rhinovirus in this study could potentially be explained by proportion of this viral species in the positive cohort.

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Respiratory viruses

COMPARISON OF TWO SYNDROMIC MULTIPLEX AND THREE MINIPLEX PANEL TESTS FOR THE DETECTION OF VIRUSES CAUSING UPPER RESPIRATORY TRACT INFECTIONS

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BACKGROUND-AIM

Infections of the respiratory tract can be caused by a diversity of pathogens. Rapid diagnosis of respiratory infections is of great importance for adequate isolation precautions and treatment. Cartridge-based multiplex panels covering numerous pathogens offer an advantage of minimal hands-on time and short time to result to commercial RT-PCR assays.

METHODS

In this study, we compared the performance of the ePlex® Respiratory Pathogen Panel 2 (RP2), the BioFire® FilmArray Respiratory Panel RP2.1 plus (BioFire RP2.1) and the four AllplexTM respiratory panels using around 100 de-identified clinical respiratory samples or virus bank samples. The performance of each test was compared against a composite reference (CR) of the three NAATs for the respective viral target. The following viral pathogens were analyzed: Influenza A and B, RSV, Parainfluenza types 1-4, Adeno-, Metapneumo-, Rhino- and Enterovirus, as well as Corona viruses 229E, NL63 and OC43.

RESULTS

The sensitivity of the three tests was comparable considering the limited amount of samples tested per target. Two targets though were an exception. Biofire RP2.1 plus detected only half the samples the other two tests detected positive for the pathogen Influenza A H1N1-2009 and Allplex completely missed to detect the pathogen Parainfluenza Type 4 which was detected by the other two tests. Specificity of the two multiplex panels was ranking from 97.6-100%, while the Allplex panels detected multiple questionable co-infections leading to lower percentages in specificity. The observed discrepancies were investigated. Most of the discrepant results have been observed in the low-pathogen-load samples.

CONCLUSIONS

The compared tests show an acceptable sensitivity taking into consideration the small sample size. In contrast, the highly complex and less simple-to-use Allplex panels demonstrated a low percentage of specificity especially for highly relevant pathogens like Influenza A, while two other tests performed excellent.

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DETECTION AND DIFFERENTIATION OF THE 4 MAIN RESPIRATORY VIRUSES WITH THE NEW SARS-COV-2/FLUA/FLUB/RSV R GENE® ASSAY

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BACKGROUND-AIM

The analytical and clinical performances of the new SARS-COV-2/FLUA/FLUB/RSV R GENE® kit that allows detection and differentiation in Nasopharyngeal swab (NPS) of SARS-CoV-2, Influenza A, Influenza B and Respiratory Syncytial Virus along with an endogenous internal control are summarized in this poster.

METHODS

Limit of Detection of each of the 4 targets was confirmed on multiple platforms (extraction: NUCLISENS® easyMAG®/EMAG®; MagNAPure 96; QIAsymphony SP; MGISP-960 and amplification: QuantStudio5/5 DX, CFX96/Opus 96) using a viral culture and/or the SARS-CoV-2WHO IS.
The LoD have been determined in TCID50 and in cp/ml through dPCR quantification.
The inclusivity of the assay has been demonstrated on 10 SARS-CoV-2 variants, 10 Influenza A subtypes, 5 Influenza B and 5 RSV strains including RSVA and RSVB.
Exclusivity and biological interferences were assessed on 40 non-targeted microorganisms and on gBlocks®.
Chemical interferences were assessed by testing endogenous (2) and exogenous (14) substances, as well as 6 transport mediums.
The competitive interference between targets was also evaluated.
Clinical performances were evaluated by comparison (clinical agreement) with BIOFIRE® FILMARRAY® RP2.1 plus through retrospective studies carried out in 4 testing sites gathering 873 NPS.

RESULTS

The following LoD were confirmed on all tested platforms:
- 380 cp/ml for SARS-CoV-2 and 250 IU/ml for 1st WHO IS SARS-CoV-2
- 1517 cp/ml for Influenza A H1N1
- 1460 cp/ml for Influenza B (Phuket)
- 276 cp/ml for RSVA

The results have demonstrated a good inclusivity on circulating strains.
Targets detection is not impacted by the presence of chemical substances, microorganisms or co-infections in NPS.
Clinical performances were shown to be comparable between SARS COV 2/FLUA/FLUB/RSV R GENE® assay and BIOFIRE® FILMARRAY® RP2.1 plus.

CONCLUSIONS

The new 5-plex assay SARS-COV-2/FLUA/FLUB/RSV R GENE® kit allows detection and differentiation, in one single reaction of the 4 main respiratory viruses involved in seasonal respiratory diseases in Nasopharyngeal swab, including a control of the specimen quality. As for the R-GENE® range of products, the kit, that is under IVDR registration, can be used on different extraction and amplification platforms.

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Respiratory viruses

DEVELOPMENT OF EIGHT SPECIFIC PRIMER SETS FOR DETECTION OF SARS-COV-2 SPIKE MUTATIONS

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BACKGROUND-AIM

On January 20, 2020, the first laboratory-confirmed case of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was reported in South Korea. SARS-CoV-2 has a single-stranded RNA genome of approximately 29.9 kb and contains various structural proteins (e.g., spike, membrane, envelope, and nucleocapsid proteins). RNA viruses have high mutation rates, with mutations in the spike gene being regularly reported. These mutations are associated with SARS-CoV-2 transmissibility, virulence, or resistance to some neutralizing antibodies. Thus, the accurate profiling of spike mutants is crucial for tracking SARS-CoV-2 variants caused by amino acid changes. In this study, eight SARS-CoV-2 spike gene primer pairs (8-SSG primer assay; 69S, 144S, 417S, 484S, 570S, 859S, 950S, and 1118S) were developed to detect spike mutations using a Sanger sequencing-based assay.

METHODS

The 8-SSG primer assay was designed based on the NC_045512.2 reference sequence between positions 21224 and 25409 using the overlap extension polymerase chain reaction method. The optimized oligonucleotide of the 8-SSG primer assay sequences was analyzed in silico by the NCBI-BLAST interface. Twenty-one respiratory pathogens (including the SARS-CoV-2 strain) were used to determine analytical specificity, and two clinical samples were used to verify the in-clinical performance.

RESULTS

The 8-SSG primer assay detected all the amino acids in the spike gene (69S: 21224 to 21930; 144S: 21843 to 22426; 417S: 22226 to 22949; 484S: 22752 to 23359; 570S: 23108 to 23811; 859S: 23680 to 24310; 950S: 24147 to 24730; and 1118S: 24647 to 25409) and showed 100% analytical specificity. Regarding clinical performance, the 8-SSG primer assay detected 52 mutants (substitution: n=40; deletion: n=9; insertion: n=3). Taken together, the 8-SSG primer assay could accurately and reliably detect SARS-CoV-2 spike mutants.

CONCLUSIONS

We developed and evaluated an 8-SSG primer assay for detecting SARS-CoV-2 spike gene mutants. The 8-SSG primer assay can be a useful tool for detecting SARS-CoV-2 spike gene mutants in a clinical setting, thereby helping profile the mutation accumulation of SARS-CoV-2.

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DISTRIBUTION OF RESPIRATORY VIRUSES DURING THE LAST YEAR OF COVID-19 PANDEMIC IN GAZI UNIVERSITY, ANKARA, TURKEY

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BACKGROUND-AIM

The epidemiyology of respiratory viruses dramaticaly changed during Covid-19 pandemic. The aim of our study is to invistigate the prevalance of respiratory viruses during last year of covid-19 ongoing pandemic.

METHODS

Nasopharyngeal swaps obtained from patients who applied to our hospital between May 2022 and April 2023 were included in the study. A total of 69817 nasopharyngeal swabs were sent to our laboratory to detect covid positivity. The age of patients was between 0-99 years. The distribution of gender was 52,4% female, 47,6% male.A total of 2598 nasopharyngeal swaps sent to our laboratory in the same year for other respiratory tract pathogen detection were included in the study. Among these samples 55.7%male, 45.6% female, aged between 0-95 Years. The Viral nucleic acid was extracted by automated system and the presence of respiratory viruses in the collected samples were detected by Real time PCR method

RESULTS

According to our results, 14,7%(10297/69817) of samples sent for SARS-CoV-2 detection was positive. The positivity rate among people who aged between 0-18 years was 7,4% (581/7864). While the patients who aged between 19-99 years showed high positivity rate with 15,6%(9716/61953). The positivity rate of samples sent to determine the other respiratory tract pathogens was 42.5%(1106\ 2598). Human rhinovirus 31.4%(348\ 1106) was detected most often, followed by Influenza viruses 23.6%(262\ 1106) and Human respriratory synctial 9%(180\ 1106). Our finding reveal that high positive rates was among patients who applied to different pediatric clinics 53%(587\ 1106). While the positivity rate among adults was 46.1%(510\ 1106).

CONCLUSIONS

Early detection of respiratory viruses can assist in managing their spread effectively. Control measures also are vital in controling the spread of these viruses. After vaccination strategies it has been observed that Covid-19 positivity has decreased compared to the first years of the pandemic. The reduction in the incidence of respiratory viruses during the pandemic also has highlited the effectiveness of control measures such as social distancing and mask use in reducing the spread of respiratory virus.

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Respiratory viruses

DIVERSITY OF RESPIRATORY VIRAL CO-INFECTIONS IN AUSTRIA DURING SEASONS 2021/2022 & 2022/2023

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BACKGROUND-AIM

During the SARS-CoV-2 pandemic, the circulation of RSV, Influenza and other respiratory viruses declined significantly due to pandemic measures and changes in behavior. In the current 2022/2023 season, a seasonally atypical re-emergence of RSV, Influenza and other respiratory viruses could be observed.

METHODS

In Austria, annual sentinel surveillance of circulating respiratory viruses is coordinated on a nation-wide level. Nasopharyngeal samples are collected year-round from over 200 sentinel physicians (including pediatricians, family doctors and hospitals) from patients presenting with acute respiratory illness and tested by PCR for SARS-CoV-2, RSV-A/-B, Influenza-A/-B/-C, Rhinovirus, hMPV and other respiratory viruses at the Center for Virology of the Medical University of Vienna.
Here, we report the incidence data for infections and co-infections with those viruses for two pandemic seasons (2021/2022 and 2022/2023) in children (0-6 and 6-18 years) and adults (>18 years).

RESULTS

In summary, the 2021/2022 season was characterized by a strong SARS-CoV-2 activity in adults, low overall Influenza activity and a moderate to high RSV-A incidence in children. In comparison, strong Influenza-A and RSV-B circulation was observed in the 2022/2023 season with only low co-circulation of SARS-CoV-2. Children under 6 years showed a more diverse pattern of viral infections and viral co-infections compared to adults. Notably, 37% of SARS-CoV-2-infected children and up to 27% of the RSV, and 26% of the Influenza-infected children were co-infected with another virus in the current 2023/2023 season. Interestingly, co-infections with Influenza + SARS-CoV-2 were less commonly observed than other combinations such as Influenza + RSV.

CONCLUSIONS

In conclusion, the unusual predominance of RSV-B as well as the overall strong respiratory virus circulation in the current 2022/2023 season warrant further epidemiological and mechanistic investigation.

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Respiratory viruses

EPIDEMIOLOGICAL TREND OF RESPIRATORY VIRUSES PRE AND POST COVID-19 PANDEMIC: A 7-YEARS ANALYSIS

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BACKGROUND-AIM

Respiratory infections have an important impact on health and are the most common reason for hospitalization in children and in immunocompromised patients. Pathogen identification is important in critical patients and the fastest and most sensitive tests used are molecular tests. In our study we considered respiratory infections separately from COVID-19 and investigated epidemiological trend over a long period with a prospective observational study. We wanted to investigate viral respiratory pathogens both in adult and pediatrics, to observe their epidemiology pre and post COVID-19 pandemic and to evaluate the number of cases in a long period, in particular from January 2016 to August 2022, in Northwest Italy, Turin.

METHODS

The study was conducted at the University Hospital Città della Salute e della Scienza di Torino, Microbiology and Virology Unit. A total of 10709 tests was performed, in particular 6424 tests were performed for pediatric population and 4285 for adult population. Two types of molecular panels were used for the analyzes: FILMARRAY Respiratory Panel and GeneXpert Cepheid Xpert® Xpress Flu/RSV.

RESULTS

In adult patients, viruses such as Adenovirus, Coronavirus (not SARS CoV), Parainfluenzavirus, Metapneumovirus and Rhinovirus/Enterovirus always presented very low positive rates. Influenza A virus and the Respiratory Syncytial virus had a peak in pre pandemic period, then a collapse during pandemic in 2020. In pediatric population Influenza A virus increased in positive rates in pre pandemic period, then decreased in 2020 and 2021 and a new increase was registered in post pandemic period (2022). Influenza B virus, Metapneumovirus and other viruses had a low positive rate. Rhinovirus/Enterovirus is consistently present in pediatric population. Respiratory syncytial virus had a constant trend with a significant increase at the end of 2021, probably resulting from the re-openings after the restrictions for SARS CoV2.

CONCLUSIONS

Our data demonstrated a change in the typical epidemiology of respiratory viruses in pre and post pandemic, in particular during 2016–2022, in a large cohort of patients and the use of a syndromic panels allowed a rapid and useful differential diagnosis of common respiratory infections in adult and children.

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Respiratory viruses

EPIDEMIOLOGY AND DIAGNOSIS OF INFLUENZA A AND B: CHANGES ASSOCIATED WITH THE COVID-19 PANDEMIC IN A NORTH-WEST ITALIAN PROVINCIAL HOSPITAL

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BACKGROUND-AIM

Since March 2020 several non-pharmaceutical interventions (NPIs) have been activated to contain the spread of SARS-CoV-2. NPIs also affected the circulation of other seasonal respiratory viruses such as influenza viruses. During the COVID-19 pandemic, our hospital and primary care facilities introduced a specific and easy protocol to promptly report all patients with Flu-like symptoms. Our study aimed to evaluate the performance of the COVID-pandemic protocol during the 2021-22 and 2022-23 Flu-surveillance periods.

METHODS

A network of primary care doctors was trained to promptly report suspected Flu-like symptomatic patients to the local public health service and to the central lab to have a nasopharyngeal swab (NPS). A molecular multiplex respiratory panel was used to simultaneously detection of influenza viruses (Flu-A, and Flu-B), respiratory syncytial virus (RSV), and SARS-CoV-2. Results were compared with the two pre-COVID Flu-surveillance periods (2017-2019).

RESULTS

We observed a dramatic reduction in Flu infections during 2020, influenced by the ongoing use of NPIs and shifts in testing priorities and surveillance systems. A total of 370 and 975 nasopharyngeal swabs were tested in Flu-surveillance 2021-22 and 2022-23 respectively. Overall, of the 1327 swabs, 38.8% were positive (media:43.9%). Flu-A positive swabs were 31.2% (media:38.7%; predominantly lineage A(H3N2)) and Flu-B positive were 7.5% (media:5.2%); lineage Victoria only). To note the absence of Flu-B positive cases in the 2021-2022 season. We tested 153 and 229 nasopharyngeal swabs, in the pre-pandemic surveillance 2017-2018 and 2018-2019 respectively. Of the 382 swabs, 35.3% were positive (media:36.3%). Flu-A positive swabs were 21.9% (media:19.6%; lineage A(H1N1)pmd09 and A(H3N2)) and Flu-B positive were 13.3% (media:16.7%; lineage Yamagata only).

CONCLUSIONS

Influenza activity recovered in 2021 and intensified during the traditional influenza winter season. The introduction of a protocol that allows a quick and simple collaboration between different actors (public health department, laboratory, primary care) and a molecular test for differential diagnosis has allowed the screening of many people, exceeding the peak in previous years.

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EPIDEMIOLOGY OF SEASONAL INFLUENZA 2022-2023 IN THE SOUTH TYROL REGION

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BACKGROUND-AIM

Seasonal influenza is an acute respiratory infection caused by influenza viruses circulating worldwide, with annual and regional variations. The seasonal trend of influenza viruses in the South Tyrol region is monitored within the "InfluNet & RespiVirNet" surveillance network, coordinated by the Istituto Superiore di Sanità-ISS. The results of the surveillance activities obtained so far during the season are here presented.

METHODS

Clinical samples have been collected from patients with flu-like syndrome. Molecular diagnosis was performed to monitor the circulation of influenza viruses as well as other respiratory viruses. Genomes of a selection of influenza viruses have been sequenced, and the HA gene sequence used for phylogenetic analysis.

RESULTS

Since the beginning of the season so far, 844 samples (17.6% of the samples analysed) tested positive for the influenza virus, among them: 545 (64.6%) belonged to A(H3N2), 91 (10.8%) were A(H1N1)pdm09 and 208 (24.6%) B/Victoria.
705 (14.7%) samples tested positive for the Respiratory Syncytial Virus (RSV, either A or B), 217 (4,5%) for Rhinovirus, 326 (6.8%) for other respiratory viruses, including SARS-CoV-2.
As expected, influenza viruses circulated more in the age group 15-24 years, followed by 6-14 years and the elderly population (>= 65 years), where type A represented >90% of the influenza viruses. 27% of the samples of paediatric patients with a respiratory infection tested positive for influenza, and 41,5%-10.3%-24.7% were the percentages for patients admitted in the wards of Medicine, Haematology and Intensive Care Unit, respectively.
Considering the co-infections with other viruses (297 samples), co-infections with influenza-SARS-CoV-2 were infrequent (7 samples), more common were the co-infection with RSV, 25 and 4 samples tested positive for influenza A or B and RSV, respectively.

CONCLUSIONS

As in other Italian regions, the 2022-2023 influenza season was characterized by an early start and several viral identifications similar to the pre-COVID-19 periods, peaking at the beginning of December (week 49). A(H3N2) was isolated mainly at the beginning of the season, while from January it was gradually replaced by B/Victoria; A(H1N1)pdm09 co-circulated simultaneously with both viruses but to a lesser extent.

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Respiratory viruses

ESTIMATING ASSOCIATIONS OF RESPIRATORY VIRUSES WITH ACUTE RESPIRATORY SYNDROMES AND DISEASES IN A 5 YEARS PERIOD IN SLOVENIA

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BACKGROUND-AIM

Acute respiratory infections (ARI) are caused by different respiratory viruses and are associated with a range of acute respiratory syndromes/diseases of various severity. Identification of causative agents of ARI has been facilitated by multiplex molecular testing. We investigated associations of respiratory viruses with acute respiratory syndromes/diseases by comparison of virological, clinical, epidemiological data.

METHODS

From national public health system weekly numbers of acute respiratory syndromes/diseases are reported to National Institute of Public Health (NIPH) as defined by International Classification of Diseases. Data on severe syndromes/diseases, specifically influenza (J10.0-J11.8), acute bronchiolitis (J21.0, J21.8, J21.9), acute bronchitis (J20.3, J20.4, J20.5, J20.6, J20.7, J20.8, J20.9), pneumonia (J12.0, J12.1, J12.2, J12.8, J12.9) were analysed. At National Influenza Centre (NIPH, National Laboratory of Health, Environment and Food) nasal/throat swabs from patients with ARI, their personal, clinical data and weekly numbers of ARI/100.000 inhabitants are collected from a sentinel of 50 primary healthcare clinics and 2 hospitals. Multiplex RT-RT-PCRs are performed to detect respiratory viruses (influenza-INF, respiratory syncytial virus-RSV, rhinovirus-RV, enterovirus-EV, coronavirus-hCoV, metapneumovirus-hMPV, bocavirus-hBoV, adenovirus-hAdV, parainfluenza virus-PIV). In total 12982 swabs were analysed. Weekly rates of reported syndromes/diseases and virus detections from 5 years prior to COVID-19 pandemic were compared for analysis (to exclude SARS-CoV-2 interference with respiratory viruses).

RESULTS

Some viruses were associated with a single acute respiratory syndrome/disease: INF with influenza; RSV with acute bronchiolitis; hCoV and PIV both with acute bronchitis. Other viruses were associated with 2 acute respiratory syndromes/diseases: hAdV, hBoV, hMPV all with acute bronchiolitis and acute bronchitis. RVs were associated with acute bronchiolitis, acute bronchitis, pneumonia (bronchiolitis was more frequent in paediatric, pneumonia in elderly patients).

CONCLUSIONS

Associations of different respiratory viruses with severe acute respiratory syndromes/diseases were determined. Results are informative to estimate the burden of different respiratory viruses in Slovenia.

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Respiratory viruses

EVALUATION OF ALLPLEX™ RV MASTER ASSAY FOR THE DIFFERENTIAL DIAGNOSIS OF RESPIRATORY VIRUSES IN THE POST-COVID ERA

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BACKGROUND-AIM

With the end of the COVID-19 pandemic, non-SARS-CoV-2 respiratory viruses are increasingly circulating, requiring differential diagnosis. A multiplex real-time PCR Allplex™ RV Master Assay has been developed as an in vitro diagnostic test for detecting major respiratory viruses in a single test. This study aims to evaluate the diagnostic performance of Allplex™ RV Master Assay.

METHODS

Allplex™ RV Master Assay can qualitatively detect SARS-CoV-2, influenza A virus (Flu A), influenza B virus (Flu B), human respiratory syncytial virus (RSV), human metapneumovirus (MPV), human adenovirus (AdV), human rhinovirus (HRV), and human parainfluenza virus (PIV). Analytical performance was verified by determining the limit of detection (LoD), reproducibility, and specificity of each target detection. The inclusivity of SARS-CoV-2 and Flu A variants was determined using in-silico analyses. Clinical performance was evaluated by comparison with other SARS-CoV-2 and respiratory virus real-time PCR assays.

RESULTS

The LoD of Allplex™ RV Master Assay was determined using plasmid DNA, as 50 copies/reaction for SARS-CoV-2 and 100 copies/reaction for other respiratory viruses. Reproducibility was confirmed between runs, sites, product lots, and operators, and the positive rates were ε95% with CV values of <5%. The high specificity was tested for cross-reactivity to 140 pathogens, and amplification and detection were identified only for the specified targets, with no interfering effects. Allplex™ RV Master Assay could also detect new variants, including recently emerged clades of Flu A H3N2 and H1N1pdm09. For the clinical performance evaluation, 944 specimens were tested, and the result showed more than 95% positive percent agreement (PPA) and negative percent agreement (NPA). The PPA of the Allplex™ RV Master Assay was 97.40%, 96.00%, 100.00%, 97.30%, 98.67%, 100.00%, 96.91%, and 96.25% for SARS-CoV-2, Flu A, Flu B, RSV, MPV, AdV, HRV, and PIV, respectively.

CONCLUSIONS

Through the validation of the analytical and clinical performance, the quality of Allplex™ RV Master Assay is valid and reliable for the detection of SARS-CoV-2, Flu A, Flu B, RSV, MPV, AdV, HRV, and PIV, enabling accurate differential diagnosis of respiratory viruses in the post-COVID era.

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Respiratory viruses

EVALUATION OF CLINICAL PERFORMANCE OF A MULTIPLEX REAL-TIME PCR KIT FOR DETECTION OF SARS-COV-2, FLU A/B AND RSV IN PATIENTS WITH SYMPTOMS OF RESPIRATORY INFECTION

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BACKGROUND-AIM

Influenza, SARS-CoV-2 and respiratory syncytial virus (RSV) cause respiratory infections with similar clinical presentation. Recently, circulation of flu and RSV has increased and co-circulation with SARS-CoV-2 was observed. Diagnostic testing is often required for optimal patient management and infection control. Multiplex real-time PCR-based tests offer the advantage of identification of infections/co-infections in a single reaction, thus providing accurate results quickly. We evaluated the clinical performance of the TaqPath™ COVID-19, FluA/B, RSV Combo Kit for detection of the 3 viruses.

METHODS

Retrospective study was performed on 438 nasopharyngeal swab samples from symptomatic patients leftover from routine diagnostic testing in the US in 2022. Evaluation of SARS-CoV-2 performance was done on 200 samples tested in parallel with TaqPath™ COVID-19, FluA/B, RSV Combo Kit and cobas® SARS-CoV-2 assay. The performance for detection of influenza A/B and RSV was assessed by testing 238 samples in parallel with the BioFire® Filmarray® Respiratory 2.1 plus Panel. Discordant samples were tested using the BioCode® CoV-2 Flu Plus Assay. Positive percent agreement (PPA) and negative percent agreement (NPA), as well as clinical sensitivity and specificity upon discordant sample resolution testing were calculated.

RESULTS

The PPA and NPA between the TaqPath kit and the comparators were: for SARS-CoV-2 94.4% and 100%; for fluA/B 98.7% and 98.1% and for RSV 92.6% and 96.8%, respectively. Ct values of the SARS-CoV-2 positive cohort spanned the dynamic range of the assays with Ct<25 (N=34%), 25δCt<30 (N=31%), and Ctε30 (N=35%). Of 77 samples showing positive concordant results for influenza, 57 were influenza A and 20 influenza B. In total 19 samples showed discordant results, and upon discordant sample resolution the clinical sensitivity and specificity of the TaqPath kit were: for SARS-CoV-2 95.7% and 100%; for fluA/B 100% and 100% and for RSV 100% and 98.1%, respectively.

CONCLUSIONS

The TaqPath™ COVID-19, FluA/B, RSV Combo Kit shows excellent performance for detection and differentiation of SARS-CoV-2, influenza and RSV infections including co-infections which is important during seasonal outbreaks when these viruses co-circulate.

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Respiratory viruses

EVALUATION OF POOLING STRATEGY FOR SARS-COV-2 REAL TIME PCR WITH SALIVA, NASOPHARYNGEAL AND SELF-COLLECTED NASAL SWAB SAMPLES

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BACKGROUND-AIM

It is important for the control of COVID-19 to test the suspected cases as early as possible and to screen the prioritized risk groups. However, shortage of diagnostic supplies in an outbreak setting presents challenges. In this study, it was aimed to investigate the effect of pooling process on the performance of the SARS-CoV-2 real time PCR test.

METHODS

Saliva (S), self-collected nasal swab (scN), and physician-collected nasopharyngeal swab (NP) samples were collected between December 2021 and April 2022. SARS-CoV-2 ORF1 a/b and pan-Sarbecovirus E gene targets were investigated with Cobas SARS-CoV-2 RT-PCR kit (Roche Molecular Systems, Inc. South Branchburg, NJ, USA). Sixty (20 S, 20 scN, 20 NP) negative (N), 62 (22 S, 18 scN, 22 NP) low positive (LP) samples (ORF1 a/b Ct mean [SD]: 30.08 [2.14]; range: 26.16-35.56) and 47 (18 S, 11 scN, 18 NP) high positive (HP) samples (ORF1 a/b Ct mean [SD]: 21.50 [3.03]; range: 16.47-25.83) were chosen. Each of these samples were mixed with four different negative samples to create pools of five samples (200 ⌈l of each sample). Two LP and two HP pools from each sample group were retested three times for intra-assay and inter-assay precision testing. In addition, two-, four-, eightfold dilutions of the pools of five LP NPS and five LP S samples were prepared and tested twice to check the highest dilution of detection.

RESULTS

Among 169 pools, 167 gave expected qualitative results for SARS-CoV-2 according to the pre-pooling results (99%). Two LP pools (one scN and NP) were found to be negative. Mean increase of 1.92 (range: 1.05-2.69) in Ct values after pooling was detected for all sample groups. Precision testing gave 100% compatible results. All NP and all HP S pools were found to be positive with all dilutions. However, among the five LP S pools; one was negative at 1/4 and one was negative at 1/8 dilutions (pre-pooling Ct values 32.32 and 31.39 for ORF1 a/b, respectively).

CONCLUSIONS

In this study, sensitivity of SARS-CoV-2 RT-PCR test with pooling of five samples was 99%. In cases with suspected COVID-19, two false negative results were obtained in LP samples after pooling. As expected, pooling reduces test sensitivity, especially among low positive samples and with larger sample pool size. Therefore, caution should be exercised when making a pooling decision.

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Respiratory viruses

EVALUATION OF THE SAVANNA RVP-4 ASSAY (QUIDEL®) FOR THE RAPID DIAGNOSIS OF VIRAL RESPIRATORY INFECTIONS

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BACKGROUND-AIM

Respiratory infections are frequent during the winter season and can be caused by several seasonal viruses. During the 2022-2023 season, co-circulations at high levels of SARS-CoV-2, Influenza (Flu) A and B viruses and Respiratory Syncytial Virus (RSV) were frequent. A rapid diagnosis enables to isolate individuals infected with one of those pathogens, and the possible administration of specific antiviral or monoclonal antibodies. We aimed to evaluate the performance of the Savanna RVP-4 (Quidel®) rapid molecular assay to detect the presence of SARS-CoV-2, Flu A, Flu B and/or RSV from respiratory samples.

METHODS

The Savanna RVP-4 is a rapid extraction and multiplex real-time PCR device, providing results in 25 minutes. We compared its results with those obtained for the routine diagnosis in our laboratory, using transcription-mediated amplification or conventional PCR assays on the Panther Fusion instrument (Hologic®). Discrepant samples were further tested on the GeneXpert® instrument (Cepheid®). The study was performed on 377 respiratory samples. 149 samples, stored at -20°C for up to 47 days, were retrospectively selected. 228 samples were prospectively included and tested with both the routine and RVP-4 assays on the same day.

RESULTS

We included a total of 47 samples positive for SARS-CoV-2, 67 for Flu A, 7 for Flu B, 89 for RSV and 33 for other respiratory viruses. There were 12 co-infections. We obtained an invalid rate with the RVP-4 assay of 6.1% for SARS-CoV-2 and 4.0% for Flu A/Flu B/RSV. When discarding invalid results, the concordance Cohen's kappa coefficient (κ) was 95.7% for SARS-CoV-2, 87.5% for Flu A/B and 99.2% for RSV. We observed 1 false positive (SARS-CoV-2) and 16 false negative results (2 SARS-CoV-2, 11 Flu A, 2 Flu B, 1 RSV). 13/16 of these discordant results were observed for samples with Ct values > 34. No cross-reactivity was observed with samples positive for Rhinovirus, Adenovirus, Metapneumovirus, Parainfluenza 1 or Parainfluenza 3.

CONCLUSIONS

Despite a lower sensitivity than conventional assays for the detection of Influenza viruses, the Savanna RVP-4 is a performant rapid molecular test. It is very easy to use and can be implemented both in laboratories and as point-of-care instruments in clinical wards.

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Respiratory viruses

EVALUATION OF TWO POINT-OF-CARE (POC) MULTIPLEX PCR'S AS A RAPID SCREENING TOOL FOR RESPIRATORY SYNDROMES

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BACKGROUND-AIM

Point-of-care (POC) molecular tests for respiratory tract diseases have the potential to improve patient management and antimicrobial stewardship. Our laboratory only offers a multiplex PCR for respiratory tract infections (RMP) in batch analysis. POC RMP can provide a solution to obtain fast and reliable results in urgent situations and low-prevalence settings. Our aim was to evaluate 2 POC RMP analyzers for their accuracy and ease of use.

METHODS

A method comparison was conducted between the RPP12 kit on the Sanity 2.0 System (Zeesan, China) and the Respiratory Panel 1.0 on the FlashDx-1000-E platform (FlashDx, China). The RPP12 kit detects 12 different pathogens (9 viral and 3 bacterial). The Respiratory Panel 1.0 detects 9 pathogens (8 viral and 1 bacterial). Only pathogens available in both assays were evaluated in this study: Flu A (FLU A) and B (FLU B), Respiratory Syncytial Virus (RSV) A and B, Adenovirus (AdV), Human Rhinovirus (HRV)/Human Enterovirus (HEV) and Parainfluenza Virus (PIV) 1, 2 and 3. Twenty-seven nasopharyngeal samples, previously positive for at least one pathogen using the AllplexTM Respiratory Panel 1A, 2, and 3 on the STARlet platform (Seegene, South Korea), were included. In addition to analytical evaluation, feasibility was assessed.

RESULTS

All samples positive for FLU A, FLU B, RSV A, RSV B, AdV and PIV were confirmed on both POC devices. The RPP12 kit additionally detected AdV (2 samples), HRV/HEV (1 sample), and PIV (1 sample), probably false positive due to fluorescence-interference. The RPP12 kit missed 5 weak HRV/HEV positive samples and the Respiratory Panel 1.0 missed 9 weak HEV/HRV positive samples. FlashDx reported higher Ct-values for all targets compared to Seegene, while Sanity 2.0 did not report Ct-values. Lab technicians rated both POC devices equally well in terms of ease of handling.

CONCLUSIONS

Both POC respiratory multiplexes are sufficiently user-friendly as rapid screening tools for respiratory syndromes. However, there are sensitivity issues with HRV/HEV detection in both assays, which may have limited clinical impact. More important, specificity problems with the RPP12 kit need to be further investigated and corrected to be useful as a screening tool. The Flashdx seems to meet the necessary requirements for accuracy, ease of use and TAT.

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Respiratory viruses

EV-D68 DETECTED IN ILI/ARI CASES REPORTED IN THE NATIONAL INFLUENZA AND OTHER RESPIRATORY SURVEILLANCE PROGRAM FROM PORTUGAL, DURING 2021/22 AND 2022/23 INFLUENZA SEASONS

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BACKGROUND-AIM

National Influenza Surveillance Program (NISP) promotes the surveillance on Influenza and other respiratory viruses, is coordinated by the Portuguese national reference laboratory for the influenza virus and other respiratory viruses (LNRVG) from the National Institute of Health Doutor Ricardo Jorge (INSA). LNRVG also integrates the European Non-Polio Enterovirus Network (EVNP) Network group.Nasopharyngeal swabs from patients that match the ARI/ILI case definition are sent form the sentinel network of primary care units, to the National Influenza Reference Laboratory for detection of influenza, SARS-CoV-2, respiratory syncytial virus (RSV) and others respiratory viruses, including enterovirus (EV) and EV-D68.This study aimed to detect EV-D68 positive samples and to perform the genetic characterization of the EV-D68 in 2021/22 and 2022/23 influenza seasons.

METHODS

1245 samples were collected during the 2021/22 and 2022/23 influenza seasons between weeks 40 (October) from the previous year to 20 (May) of the next year. Enterovirus (EV) laboratory diagnosis was performed by real time multiplex RT-PCR, Allplex™ Respiratory panel and confirmation of EV-D68 was performed by in-house real time RT-PCR. Partial VP1 region was sequenced according to Nix et al. (2006) and Savolainen et al. (2002) protocols.

RESULTS

Enterovirus was detected in 1,9 % (24/1245) of the samples. The EV-D68 was confirmed in 42% (10/24) of the EV positive cases, mainly during the winter months (between November and January). EV-D68 cases were aged between 9 and 66 years old; the majority (78%) (7/9) were older than 15 years old (1 is Unknown). 70% (7/10) were female. Co-infections with one more respiratory virus were detected in 9 EV-D68 cases.

CONCLUSIONS

EV-D68 was detected in ILI/ARI cases at primary care health units, mainly adult population. The detection of EV-D68 in adults highlight the need of further studies to understand the role of this population in the transmission of this virus to children, that are in risk for severe disease. Co-infections with other respiratory viruses were frequently detected. The specific diagnosis of EV-D68 infections is essential to manage and prevent outbreaks.
Further studies and an active surveillance are required to better understand the epidemiology of EV-D68 in Portugal.

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Respiratory viruses

FACILITATING INTER-LABORATORY COMPARISON THROUGH THE STANDARDISATION OF RESPIRATORY VIRUS MOLECULAR ASSAYS USING DDPCR

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BACKGROUND-AIM

Viral pathogens are the most common cause of respiratory tract infection across all age groups. In severe cases, identifying the causative agent aids targeted treatment and can lead to improved clinical outcome.
Molecular methods including multiplexed assays have improved detection of viral pathogens. However, the lack of International Standards for respiratory viruses means it can be difficult to compare accuracy and reliability of molecular respiratory assays from one laboratory to another. Digital droplet PCR (ddPCR) allows for reliable quantitation of quality control materials in the absence of International standards and supports inter-laboratory comparison, method verification and validation.
This study's aim was to assess standardisation of viral load quantitation of Influenza A (INFA), Influenza B (INFB), Respiratory Syncytial Virus A (RSVA) and SARS-CoV-2 (SCV2) through the use of ddPCR within a single multiplexed format.

METHODS

Characterised clinical isolates of the four viral targets were serial diluted in suitable matrices. The series were initially assessed using individual real-time qPCR assays to establish titres and linearity. Selected titres for each viral pathogen were combined into single vial format, further characterised using real time qPCR and subsequently, characterised using ddPCR (BioRad QX200) to establish calibrated measurements in ddPCR copies/ml.

RESULTS

Initial qPCR showed variable viral load across different molecular assays depending on the type of viral target or calibrator, and/or assay used. Characterisation using ddPCR and the use of the data to normalise qPCR values resulted in harmonisation of standard curves and equivalent quantitation. This suggests that ddPCR can be used to aid standardisation of PCR workflows for viral load determination.

CONCLUSIONS

Comparison of inter-laboratory data and the validation of a respiratory molecular assay prior to its clinical application can pose challenges due to the absence of International Standards. Characterisation and testing using qPCR alone showed results dependant on aspects such as type of calibrator used. Digital PCR allows for calibration in the absence of a standard, which facilitates inter-laboratory comparison and implementation of the assay into the clinical laboratory setting.

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Respiratory viruses

HIGH DETECTION RATES OF SARS-COV-2, INFLUENZA A, INFLUENZA B, AND RSV WITH THE MULTIPLEX ALINITY M RESP-4-PLEX ASSAY

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BACKGROUND-AIM

In a joint statement, WHO and ECDC emphasized the importance of monitoring SARS-CoV-2, influenza and RSV to better understand the impact of co-circulation of respiratory viruses and to strengthen prevention and control measures. Thus, the objective of this study was to evaluate the accuracy of the Alinity m Resp-4-Plex assay with regard to detection and differentiation of the respiratory viruses SARS-CoV-2, influenza A, influenza B and RSV in comparison to another on-market assay (Allplex SARS-CoV-2/Flu-A/Flu-B/RSV).

METHODS

Following initial testing with Alinity m Resp-4-Plex or with Allplex SARS-CoV-2/Flu-A/Flu-B/RSV, leftover de-identified patient samples were retested with the other assay as follows: 300 samples negative for all 4 pathogens, 201, 200, 73, and 189 samples positive for SARS-CoV-2, influenza A, influenza B, and RSV, respectively. Samples were categorized according to their Alinity m Ct values: <25; 25-30; 30-35; >35.

RESULTS

In the Alinity m Ct-categories <25; 25-30; 30-35 and >35, Allplex detected SARS-CoV-2 in 53/53; 44/48; 22/51 and 2/49 samples, respectively, with higher Ct-values and partially only positive for one of three genes. For influenza A, the recovery rates were 83/84; 43/51; 25/37 and 3/26; for influenza B 35/37; 5/19; 2/9 and 0/8; and for RSV 21/21; 34/34; 31/55 and 6/79, respectively. Overall detection rates were 60%, 78%, 58%, and 49% for the four respiratory viruses, respectively. Two influenza A samples pretested positive with Allplex could not be confirmed by Alinity m Resp-4-Plex. All 300 negative samples by Alinity m Resp-4-Plex were also tested negative by Allplex.

CONCLUSIONS

In this comparative evaluation of Alinity m and Allplex using a large number of clinical samples positive for either one of the four respiratory viruses and falling into different Ct categories (n=663), Alinity m Resp-4-plex showed considerably higher detection rates compared to Allplex SARS-CoV-2/Flu-A/Flu-B/RSV and a specificity of 100%. Discordant results between the two methodologies could be due to additional freeze/thaw cycles of specimens prior to testing. Nevertheless, the higher sensitivity of the Alinity m Resp-4-Plex assay remains obvious.

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Respiratory viruses

IMPLEMENTATION OF NEUMODX FLU A-B/RSV/SARS-COV-2 ASSAY INTO ROUTINE MOLECULAR CLINICAL LABORATORY IN A HOSPITAL SETTING

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BACKGROUND-AIM

After the reoccurrence of influenza in winter season 2021-2022, demand for FluA-B and RSV testing of patients treated in the emergency room (ER) and hospital departments increased. Shorter turnaround times (TATs) were required. Herein we evaluate the performance of NeuMoDx Flu A-B/RSV/SARS-CoV-2 Assay (NeuMoDx 4-plex) for detection of influenza A-B, RSV and SARS-CoV-2 in a hospital setting.

METHODS

LightMix Modular Assays are the reference method for detection of FluA-B, RSV and SARS-CoV-2. A total of 93 nasopharyngeal swabs (NS) positive for influenza A (n=26), influenza B (n=18), RSV (n=18), SARS-CoV-2 (n=24) and negatives (n=13) were tested with NeuMoDx 4-plex and compared with the reference method. Nonconforming samples were subsequently analyzed using Xpert® Xpress SARS-CoV-2/Flu/RSV. Additionally, 14 NS positive for other circulating respiratory viruses (including Adv, hMPV, PIV1-4, hRV, hEV, OC43, NL63 and HKU1) were tested with NeuMoDx 4-plex to rule out cross reactivity. Turnaround time (TAT) was determined as the interval between the time of sample reception and the reporting of the final results.

RESULTS

Comparison of NeuMoDx 4-plex against the reference method showed 96.2% (25/26) sensitivity for FluA and 100% (18/18, 18/18 and 24/24) for FluB, RSV and SARS-Cov-2. Specificity was 98.5% (26/25) for FluA, 98.7% (19/18) for FluB, and 100% (18/18, 24/24) for RSV and SARS-CoV-2. FluA and FluB were detected in 2 nonconforming samples, which went undetected by the reference method. FluA was missed by the NeuMoDx 4-plex in 1 sample. No cross reactivity was detected with the novel method in 14 tested swabs. TAT for the reference method and NeuMoDx 4-plex was 160 and 104 minutes, respectively.

CONCLUSIONS

With implementation of NeuMoDx 4-plex into routine molecular clinical laboratory, we significantly reduced TAT for samples received from ER and hospital departments. The novel method demonstrated reliable results and increased sensitivity when compared to reference method. Due to the fully automated procedure of NeuMoDx System, the hands on time is reduced, as well the probability of human error.

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Respiratory viruses

INFLUENZA VACCINATION COMPLIANCE BEHAVIORS OF PHYSICIANS IN TURKEY

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BACKGROUND-AIM

This study has aimed to investigate the compliance/non-compliance behaviors of physicians against influenza vaccination in Turkey and the factors acting on it. Depending on the outcomes of the research, the study also aimed to focus on the contribution to increasing the vaccination ratios among physicians within the current COVID-19 pandemic conditions.

METHODS

The Chi-square and Kruskal-Wallis tests were conducted to examine whether there is a statistically significant difference between physicians' compliance/non-compliance to the influenza vaccination and the factors behind having vaccinated regularly. Multiple logistic regression was employed to investigate the underlying factors such as age, gender, professional experience, living with risk groups for influenza, perceived risk groups, knowledge, and attitudes to influenza vaccinations of vaccination compliance among physicians. All analyses were employed using Statistical Package for Social Sciences version 21 (IBM SPSS) software.

RESULTS

Among the 301 physicians only 82(27.2%) have had the 2019-2020 seasonal influenza vaccination and only 86 physicians (21.2%) were vaccine compliant. Based on the descriptive statistics, the majority of the participants who were vaccination compliant were female (61%), were aged 50 years and over (26%), and had 15 years and over professional experience (44%). Using multiple logistic regression analysis, we found no effect of sample population characteristics, and perceived risk groups on the vaccination compliance of physicians. The results showed that having seasonal influenza vaccination in 2019-2020, diagnosing influenza in 2019-2020, living with specific risk groups, knowledge about influenza vaccination, and attitudes to influenza vaccination have varying effects on the vaccination compliance of the physicians.

CONCLUSIONS

The vaccination rate of HCWs was found to be too low, and urgent action is needed to be taken to vaccine compliance and followed up.

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Respiratory viruses

INFLUENZA-LIKE-ILLNESS IN PEDIATRIC PATIENTS ACCESSING THE EMERGENCY ROOM DURING WINTER SEASON 2022/2023

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BACKGROUND-AIM

Our aim is to evaluate, in the current winter season, the spread of the main viruses responsible for ILI in pediatric patients, with the exclusion of SARS-CoV-2.

METHODS

Between 12 October 2022 and 12 May 2023, in patients with respiratory symptoms accessing the Pediatric Emergency Room (ER) at the Sant'Orsola Polyclinic of Bologna, naso-pharyngeal (NP) swabs were collected for the detection of: Influenza A (FLU-A), Influenza B (FLU-B), Respiratory Syncytial Virus (RSV), Rhinovirus (HRN), Parainfluenza Virus (PIV), Adenovirus (ADV) and Metapneumovirus (MPV). The samples were processed using the AllplexTM RV Essential Assay (Seegene MuDTTM technology, South Korea).

RESULTS

During the study period, 1675 NP swabs were analysed. A positive result, for at least one of the viruses responsible for ILI, has been found in 1378 samples (82%). In particular, positivity was observed as follows: 389 (28%) FLU-A, 98 (7%) FLU-B, 424 (31%) ADV, 277 (20%) RSV, 376 (28%) HRN, 111 (8%) MPV, 65 (5%) PIV. A coinfection was found in 329 cases (24%).
The first FLU-A positive sample was detected in the 41st week of 2022 and the peak was reached in the 48th. At the end of the 19th week of 2023, the number of positive samples was almost zero.
The first FLU-B positive sample was detected in the 46th week of 2022. The peak was reached between the 8th and the 10th week of 2023 and by the 19th week only rare cases were observed.
The first RSV positive sample was detected in the 46th week of 2022, while the peak was in the 49th week; by the 19th week of 2023 there are no cases of infection.
Between the 12th and the 13th week of 2023, a peak of ADV has occurred and it has not been decreased yet.
The 80% of RSV positive children was less than two years old, in comparison to the 53% of ADV positive children and to the 50% of FLU-A positive children.

CONCLUSIONS

The use of the multiparameter molecular test with results available in few hours, allowed to simplify the diagnostic path used in children with ILI accessing the ER. If the patient has a clinical suspicion of viral respiratory infection, no further diagnostic tests is performed. Only in case of negative result with the persistence of symptoms, further diagnostic investigation should be carried out.

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Respiratory viruses

INVESTIGATION OF THE EFFECT OF IMMUNE SUPPRESSING CONDITIONS ON PROLONGED PCR POSITIVITY IN PATIENTS DIAGNOSED WITH COVID-19

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BACKGROUND-AIM

SARS-CoV-2 infection may lead to different clinical manifestations and chronic outcomes in healthy and immunosuppressed individuals.People who are immunosuppressed are thought to spread SARS-CoV-2 longer, increase the duration of viral transmission and act as a reservoir for potential mutations.A better understanding of prolonged PCR positivity in this patient group is very important both from a therapeutic and public health perspective.The aim of our study is to examine the effect of immunosuppressive conditions on prolonged PCR positivity in patients diagnosed with COVID-19.

METHODS

The clinical data, SARS-CoV-2 PCR test results, viral load values of 42 patients from the Adult Hematology and Bone Marrow Departments who had SARS-CoV-2 PCR positivity for more than 14 days were included in our study.The samples of these patients who came to our laboratory were studied using the real time PCR method.

RESULTS

Our study included 42 patients 19(%45,2) male and 23(%54,7) female, between the ages of 21 and 93.Except for one patient(thalassemia majör) the other patients(such as acute myeloid leukemia, multiple myeloma, chronic lymphocytic leukemia) had a diagnosis of hematological malignancy.All patients diagnosed with malignancy(97.6%) were receiving immunosuppressive therapy.About half of the patients(%45,2) included in our study had received a stem cell transplant.Patients have prolonged COVID-19 positivity ranging from 14 to 78 days.The CT(Cycle Threshold)values of SARS-CoV-2 PCR tests of the patients ranged from 10 to 34.

CONCLUSIONS

In our study it was observed that patients with COVID-19 positivity for more than 1 month had more severe symptoms at the time of admission to the hospital.In addition, it was determined that there were more stem cell transplant patients in this group compared to the patient group with prolonged COVID-19 positivity for 14-30 days.The immunosuppressive patient group is at risk for persistent PCR positivity migrating radiographic findings respiratory symptoms and systemic symptoms.In this group especially in patients with hematological malignancies, immunosuppression caused by the primary disease as well as myelosuppression and lymphopenia caused by the treatments given are thought to adversely affect the prognosis of COVID-19.

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Respiratory viruses

LIGAND-BASED DISCOVERY OF CORONAVIRUS MAIN PROTEASE INHIBITORS USING MACAW MOLECULAR EMBEDDINGS

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BACKGROUND-AIM

Ligand-based drug design methods are thought to require large experimental datasets to become useful for virtual screening. In this work, we propose a computational strategy to design novel inhibitors of coronavirus main protease, Mpro.

METHODS

The pipeline integrates publicly available screening and binding affinity data in a two-stage machine-learning model using the recent MACAW embeddings. Once trained, the model can be deployed to rapidly screen large libraries of molecules in silico.

RESULTS

Several hundred thousand compounds were virtually screened and 10 of them were selected for experimental testing. From these 10 compounds, 8 showed a clear inhibitory effect on recombinant Mpro, with half-maximal inhibitory concentration values (IC50) in the range 0.18–18.82 lM. Cellular assays were also conducted to evaluate cytotoxic, haemolytic, and antiviral properties.

CONCLUSIONS

A promising lead compound against coronavirus Mpro was identified with dose-dependent inhibition of virus infectivity and minimal toxicity on human MRC-5 cells.

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Respiratory viruses

MOLECULAR CHARACTERIZATION OF RESPIRATORY SYNCYTIAL VIRUS IN CHILDREN LESS THAN 5 YEARS FROM INDIA

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BACKGROUND-AIM

RSV is the major cause of severe LRTI in children. It has 2 subtypes: RSVA and B, both having several genotypes. This study was undertaken to detect the genotypes of RSV circulating in our region during 2019, 2021, and 2022 and to determine the association of RSV subtype with severity.

METHODS

Study includes children δ5 years admitted in JIPMER with SARI. Subtyping was done by Real time PCR following which the 2nd hyper variable region of G gene was sequenced using ABI3500. Sequences were analysed using MEGAv11, and phylogenetic tree was build using Neighbour Joining Method, with a bootstrap of 1000.

RESULTS

Total no of children were 500, whose mean age was 15.8 months, and majority were males (65.6). In 2019 and 2021, RSVA was predominant, while in 2022 it was RSVB. Clinical severity score (CSS) was calculated and we observed that severe cases (ε3) was significantly associated with RSVA. Phylogenetic analysis showed RSVA clustering around GA2.3.5 and RSVB around GB5.0.5a. A total of 57 amino acid variations for RSVA and 25 for RSVB were observed. Novel variations also were observed in this study, 7 for RSVA (L265P, L266P, T245N, T264I, P276L, G272D, S277L) and 7 for RSVB (K258G, T280S, Q283R, N296S, T302A, E305G, S309L).

CONCLUSIONS

While recent study from north eastern states revealed both A and B to be co-circulating in equal proportion, in our study RSVA was the major circulating subtype in 2019 and 2021, while in 2022 RSVB replaced RSVA. RSVA showed significant association with clinical severity and mortality. Globally, RSVA GA2.3.5 has been reported since 2010-2016 and RSV B GB5.0.5a since 2013-2014. Studies have shown that amino acid substitutions are important for the development of antiviral immunity and their variability may be shaped by host immune pressure. We observed a flip -flop substitution (L274P) which was previously associated with the decreased affinity of specific neutralizing antibodies by Krivtskaya et al. Amino acid substitution at 258 and 266 positions (within mucin like region2) have been associated with immunogenic and antigenic properties by W.Li,et al. In our study we also observed both known and novel variation at 266th position. This study has provides a baseline data from our region, which can be used for further studies.

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Respiratory viruses

NON-SARS-COV-2 RESPIRATORY VIRUSES CO-DETECTION IN SPECIMENS COLLECTED FROM CHILDREN ADMITTED TO THE BAMBINO GESù CHILDREN'S HOSPITAL IN ROME OVER A PERIOD OF 1 YEAR

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BACKGROUND-AIM

Respiratory viruses other than severe acute respiratory syndrome coronavirus 2 continue to represent a significant burden of disease. Although respiratory virus co-detections are common, analysis of combinations of co-detected viruses is not fully investigated. The aim of this study is to analyse the epidemiology of non-SARS-CoV-2 respiratory viruses and the viral pairings occurring in clinical specimens collected from children admitted to the Bambino Gesù Children's Hospital in Rome over a period of 1 year.

METHODS

Between January 2022 and January 2023, a total of 6969 specimens were screened for respiratory viruses using a multiplex PCR respiratory panel. Respiratory samples were collected and retrospectively analysed from a total of 3704 children with a median age of 2.4 years (interquartile range: 0.5-6.8).

RESULTS

Of 6969 respiratory samples, 58.3% were positive for at least one virus. Of these, viral mono-infection was found in 71.0% (N=2886/4062) while co-infection was found in 28.9% (N=1176/4062) specimens. In particular, co-detection mainly involved two viruses (71.5%, N=841/1176) while the detection of three or more viruses was less frequent 21.2% (N=250/1176) and 7.2% (N=85/1176), respectively. Overall, in samples with viral mono-infection, rhinovirus (HRV) was the most frequently detected pathogen 47.4% (N=1387/2886), followed by respiratory syncytial virus B (RSV B) 10.0% (N=289/2886) and influenza virus A 6.9% (N=198/2886). By analyzing combinations of co-detected respiratory viruses the most frequently observed pairs involved mainly HRV detected with adenovirus (AdV; 7.6%, N=89/1176), or RSV B (6.8%, N=80/1176), or human enterovirus (HeV; 6.0%, N=71/1176) or human bocavirus (HBoV; 5.7%, N=67/1176). However, although the wide dissemination of HRV, the viruses mainly detected in viral co-infections were HeV (74.1%, N=240/324), HBoV (73.1%, N=311/423) and AdV (71.8%, N=275/383).

CONCLUSIONS

The use of a multiplex PCR respiratory panel is useful to better understand the role of co-infections. Further studies are required to confirm the significance of the viral pairings observed in this study and to elucidate the virologic mechanisms underlying the association that may lead to cooperation or competition between co-detected viruses.

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Respiratory viruses

PHYLODYNAMICS OF SARS-COV-2 IN PIEDMONT, NORTH-WESTERN ITALY

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BACKGROUND-AIM

SARS-CoV-2 pandemic changed the approach to investigation of respiratory viruses. From early 2020, the efforts for massive sequencing increased to characterize the changes of this novel virus, either from a biological (e.g. mutation rate, recombination, lineages) or from a clinical point of view (e.g. infectiousness, pathogenicity, vaccine and development and escape, resistance to antiviral therapies). In Piedmont, a region in North-Western Italy, massive sequencing of SARS-CoV-2 started in late 2020, and up to date more than 6000 sequences were collected and shared to national and international (GISAID) databases. Here we present the phylodynamic analysis produced as a result of the SORGENTE project (SORveglianza GEnomica in PiemoNTE, genomic surveillance in Piedmont).

METHODS

We downloaded from GISAID 6115 sequences and metadata from Piedmont (collection date 01/01/2020- 31/12/2022). We selected 10% of the sequences stratified by month. The S gene sequences were aligned based on the amino acid sequence. The results of bModelTest and path sampling were used as prior information in BEAST2 software, together with province and date of collection for phylodynamic analysis.

RESULTS

BEAST2 estimated Torino province as the most probable location of origin for the root and for both Delta and Omicron clades. Two clades localized in Novara and Alessandria probably had origin form Novara, a province close to Lombardy, where SARS-CoV-2 had a large impact.
The tMRCA at the root was estimated to mid 2019,while for the Omicron clade was estimated at 2022. Delta clade includes also some of Alpha and B.1 sequences, thus its tMRCA is estimated earlier than the first comparison of Delta variant (2020.5).

CONCLUSIONS

The spread of SARS-CoV-2 from Torino is a realistic framework, since the first cases were detected in Torino in late February 2020, and, being the chief town of the region, it has more people transfer compared to the rest of the area. Also the Novara province may have had multiple entrance, since it has many contacts with Lombardy and Milan area.
Also the tMRCA of the root is in line with other studies, that detected traces of the virus in 2019 in wastewaters and stored human samples.

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Respiratory viruses

REAL-TIME CELLULAR ASSAY FOR MONITORING OF CLINICAL SARS-COV-2 SUSCEPTIBILITY TO PROTEASE INHIBITORS

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BACKGROUND-AIM

With 6.9 million deaths to date, the recent pandemic has highlighted the need for effective therapies against SARS-CoV-2. Monoclonal antibodies, used mainly in the last two years, became largely ineffective after the emergence of Omicron. The development of 3CL protease inhibitors (PIs) such as Paxlovid (nirmatrelvir/ritonavir) has been a real breakthrough, regardless of variants. Widely used worldwide, they are recommended in France as first-line treatment to prevent the risk of hospitalisation. However, their increased use also increases selection pressure and therefore the risk of resistant variants emerging. Analysis of GISAID database suggests that resistance to PIs is currently rare but real-time phenotypic and genotypic monitoring remains essential to predict the emergence of new resistant mutations. The aim of this project is to evaluate the susceptibility of clinical SARS-CoV-2 strains to Paxlovid using Real Time Cellular Analysis (RTCA) and compare it to a standard antivirogram (ATV).

METHODS

Omicron and Delta strains were inoculated at 0.1 MOI onto VERO E6 TMPRSS2. In accordance with local epidemiology, a total of 30 SARS-CoV-2 isolates were cultured from samples collected from hospitalised patients. Six concentrations of Paxlovid were tested, from 1500nM to 5nM, associated with Pgp inhibitor at 2µM. In RTCA, cell viability is measured continuously for 72 hours using Agilent xCELLigence®, while a neutral red stain is used at 72 hours for standard ATV. The susceptibility of each strain is based on the half-maximal inhibitory concentration (IC50) determined from dose-response curves.

RESULTS

The median IC50 of the Omicron and Delta strains determined by RTCA were 25nM (±2,6nM) and 33nM (±28nM) respectively, compared to 49nM (±52nM) and 89nM (±17nM) for standard ATV. No significant differences were found between variants or techniques. In addition, the standard deviations of the control strain, 44nM for RTCA and 190nM for ATV, suggest less variability in RTCA, which would be more accurate.

CONCLUSIONS

In conclusion, RTCA gives similar results to standard technics and existing literature. This real-time assay is less time-consuming, a significant advantage for BSL3 pathogens, and could be used routinely to monitor the emergence of resistance to PIs and adapted to other antivirals.

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Respiratory viruses

RE-EMERGENCE OF RESPIRATORY SYNCYTIAL VIRUS AFTER THE ALLEVIATION OF NON-PHARMACEUTICAL INTERVENTIONS DUE TO THE SARS-COV-2 PANDEMIC

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BACKGROUND-AIM

Respiratory syncytial virus (RSV) represents one of the most important respiratory pathogens affecting all age groups. Following the extensive non-pharmaceutical interventions (NPIs) and societal behavioral changes of 2020 and 2021 in wake of the SARS-CoV-2 pandemic, an interseasonal rise in cases was observed in the summer and fall of 2021.

METHODS

The aim of this study was to characterize the local molecular epidemiology of RSV infections in the season of 2021/2022 to the three pre-pandemic seasons by sequence analysis of the complete G gene. Additionally, clinical data were retrieved from patient charts to determine the clinical significance of RSV infections of the same period.

RESULTS

The peak of RSV detections occurred in calendar week 40 of 2021 (September/October), 18 weeks before the usual peak observed in the three pre-pandemic seasons. The sequence analysis of 181 RSV-A and 144 RSV-B strains revealed a close phylogenetic relatedness with assignment to the same genotype regardless of the season of origin. With 88.9% of all cases, a significantly higher amount of pediatric cases ($p<0.001$) was observed for season 2021/2022 with positivity rates of up to 48.9%. Most of the assessed clinical parameters were similar in comparison to the three pre-pandemic seasons. For the pediatric cases, significant differences were observed for an increased number of siblings in the household ($p=0.004$), a lower rate of fever ($p=0.007$), and a reduced amount of co-infections ($p=0.001$). Although the mean age of the adult patients was significantly younger (47.1 vs 64.7, $p<0.001$), high rates of comorbidities, lower respiratory tract infections, and ICU admissions prevailed.

CONCLUSIONS

The NPIs in wake of the SARS-CoV-2 pandemic had a tremendous impact on the epidemiologic characteristics and seasonality of RSV. The continued effect of behavioral changes on the circulation of respiratory viruses as well as the potential implementations of new treatment strategies warrant further epidemiologic studies of this important pathogen.

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Respiratory viruses

RESPIRATORY INFECTIONS DURING THE POST-PANDEMIC PERIOD IN NORTHERN GREECE

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BACKGROUND-AIM

Background-Aim: Respiratory infections are among the most common causes of morbidity, especially during winter time worldwide. As the clinical presentation of these infections is similar and differential diagnosis is sometimes difficult, early laboratory investigation and diagnosis is very important, in order for the patients to be treated appropriately and on time. The aim of the present study is to detect the presence of respiratory viruses in patients with respiratory infection during the post-pandemic winter period 2023 in Northern Greece.

METHODS

Methods: Sixty nasopharyngeal swabs were obtained from patients with Influenza-like symptoms, sent to National Influenza Reference Center of Northern Greece in the context of epidemiological surveillance of EODY during the months of February-April 2023. The age range was 10-81 years with a median age of 48 years, (63.30% Women / 36.70% Men). DNA extraction was performed using the MagMAX™ Viral/Pathogen Kit (Thermo Fischer Scientific) while virus detection was performed with the FTD SARS-CoV-2/FluA/FluB/HRSV and FTD kits Pathogen 21 kit from Siemens Healthineers with the real time RT-PCR method. Both procedures were carrying out according to the manufacturer's instructions.

RESULTS

Results: According to the results, the SARS-CoV-2 was detected in 10 samples (16,7%), influenza virus in 9 samples (15%), out of which 2 belonged to type A and 7 to type B, and RSV virus in one sample (1,7%). Moreover, human coronaviruses (229E, HKU-1, NL63) were detected in 8 samples (13,3%), Human Adenovirus (hAdV), in 2 samples (3,3%), EnteroVirus (EV) in 2 samples (3,3%), HPVI-3 in 4 samples (6,7%), while 4 samples (6,7%) were positive for hMPV (A and B types). 3 co-infections were observed, one between SARS-CoV-2/hAdV viruses and the second between SARS-CoV-2/ EVs. The third one was between Influenza B type/ EVs. No statistical difference was reported in the presence of the mentioned viruses regarding the age and gender of the patients (page=0,512 pgender=0,614).

CONCLUSIONS

Conclusions: During the post-pandemic period and the lifting of restrictive measures, all known respiratory viruses returned to circulation, making their laboratory investigation necessary for the best and timely treatment of patients.

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Respiratory viruses

RESPIRATORY SYNCYTIAL VIRUS SUBTYPE-A AND -B GENETIC DIVERSITY AND ITS IMPACT ON BRONCHIOLITIS SEVERITY BEFORE AND AFTER PANDEMIC RESTRICTIONS IN ROME

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BACKGROUND-AIM

After pandemic restrictions were lifted, there was a surge of respiratory syncytial virus (RSV) hospitalization in autumn 2021, and a large number of cases also in the winter season 2022/23. In this study, we aimed to characterize viral diversity of RSV-A and -B causing bronchiolitis in Rome, also comparing clinical data, before and after the COVID-19 pandemic.

METHODS

From 2017/18 to 2022/23, 263 RSV-positive samples, prospectively collected from infants hospitalized for bronchiolitis in the Pediatric Emergency Department, and in the pediatric intensive care unit (PICU), Sapienza University of Rome, were sequenced in the second-half of the G gene. Phylogenetic results, amino acid substitutions and predicted glycosylation patterns were analyzed and patients' data were compared.

RESULTS

Predominance of RSV-A and -B alternated in the study seasons; RSV-A dominated in 2017/18 (69%), 2019/20 (89%) and 2021/22 (74%) whereas RSV-B was predominant in 2018/19 (77%) and 2022/23 (72%). According to the phylogenetic analysis, RSV-A sequences, all ON1 genotype, were quite distant from the ancestor; two divergent clades included sequences from the pre- and post-pandemic seasons. Nearly all RSV-B were BA10 genotype; interestingly, a divergent clade of 2021/22 and 2022/23 sequences, was characterized by the aa substitutions P216S, P223L, and K258N that introduced a N-glyc site. Moreover, 14 sequences acquired a further predicted N-glyc site with the change K209N.
Comparing all study seasons, 2021-2022 RSV-A cases had lower need of O2 therapy and of intensive care whereas RSV-B infected infants were more frequently admitted PICU and needed O2 in 2022-2023.

CONCLUSIONS

These data show that the increased number of total RSV cases observed in 2021/22 was not associated with a single more virulent RSV strain but driven by RSV-A phylogenetically related to pre-pandemic strains; hence, more hospitalizations were likely due to a waning population immunity. The evolutionary divergence observed in post-pandemic RSV-B strains could be associated to higher bronchiolitis severity in 2022/23. Extensive data on RSV molecular epidemiology are needed to associate patterns of genomic diversity with transmissibility and virulence or with resistance to therapeutics.

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Respiratory viruses

RESPIRATORY VIRUSES INTEGRATED SENTINEL SURVEILLANCE IN LOMBARDY (NORTHERN ITALY), FROM AUGUST 2022 TO MAY 2023

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BACKGROUND-AIM

After the Covid-19 pandemic, the urgent need to develop and sustain resilient population-based integrated surveillance system for influenza, Covid-19 and other respiratory virus infections (such as RSV or new viral diseases of public health concern) became compelling. We report the results of the integrated sentinel surveillance for respiratory viruses of influenza-like illness (ILI) in Lombardy (Northern Italy) from August 2022 to May 2023

METHODS

As the regional reference laboratory of ILI surveillance network (InfluNet&RespiVirNet), we analysed 2,428 nasal-pharyngeal swabs (NPSs) collected by sentinel physicians from as many ILI outpatients in Lombardy from August 1st, 2022 to May 5th, 2023. Differential diagnosis was carried out by specific real-time PCR assays to detect influenza viruses (IVs), SARS-CoV-2, respiratory syncytial virus (RSV), metapneumovirus (MPV), parainfluenza viruses (PIV), rhinovirus (RV), enterovirus (EV), parechovirus (HPeV) and adenovirus (AdV)

RESULTS

Overall, in 84.5% of NPS at least one virus was identified. IVs were detected in 23.2% of NPSs: 63.2% of these were A(H3N2), 12.9% were A(H1N1)pdm09 and 23.9% were IV B/Victoria lineage. A(H3N2) IV were detected mainly in school-age children (5-14y) and adults (15-64y) with an epidemic peak in November/December 2022, followed by an increase of IV-B detection that peaked in February/March 2023. SARS-CoV-2 was identified in 5% of NPSs mainly collected from elderly (>64y), circulated in all the study period. RSV was identified in 9% of NPSs collected from ILIs mainly aged 0-4 years from November 2022 to February 2023. 20% of ILI were RV-positive and were identified mainly in the young children (0-4y) during all the study period. AdV was identified in 14% of NPSs mainly collected from paediatric population (0-14y) with two epidemics (August/September 2022 and April/May 2023). MPV and PIV were identified in 7% of ILIs. PeV was detected in 3% of NPSs all collected from children 0-4 years, EV was identified in 7% of paediatric (0-14y) ILIs; EV/PeV circulated from August 2022 to February 2023

CONCLUSIONS

ILIs integrated surveillance allows the monitoring of the community transmission of viral respiratory infections by uncovering changes in their epidemiological features and helping in clinical management of these infections

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Respiratory viruses

RESURGENCE OF RSV AND PARAINFLUENZA VIRUS TYPE 3 AFTER RELAXATION OF ANTI-COVID 19 RESTRICTIONS IN TAIWAN, 2022

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BACKGROUND-AIM

Anti-COVID 19 mitigation measures intensely perturbed the circulation and spreading of respiratory syncytial virus (RSV). A local RSV epidemic surged in Taiwan since October 2022 after the relaxation of COVID-19 restrictions. This study aimed to delineate the clinical picture and virological features of this outbreak.

METHODS

We prospectively enrolled 105 hospitalized children with positive RSV rapid tests between October and December 2022 at two community-based hospitals. Oropharyngeal swabs were obtained for molecular testing for RSV and parainfluenza virus (PIV) type 1-3. RSV genotype is determined by sequencing RSV G gene ectodomain and phylogenetic analysis is carried on with available GenBank sequences. Clinical characters were compared and analyzed by using SPSS software.

RESULTS

RSV-B (99/105, 94.2%) accounted for this RSV outbreak in Taiwan after easing the anti-COVID-19 measures. The genotype is belonged to BA9 and is distinct from previous local circulating strains. Two RSV BA9 clusters are found and genetically close to the USA strain in 2022 (OP890348.1) and an Australia strain (OM857377.1), respectively. This RSV outbreak is associated with the co-emergence of PIV3, and 45 of 105 (42.8%) cases have PIV3 co-infection. A shift in age of involved cases was seen and the median age of RSV infections was 21.5 months. PIV3 co-infection didn't affect clinical severity and treatment. The median hospitalization and febrile duration were 6.0 and 2.0 days, respectively.

CONCLUSIONS

RSV activity plummeted to an unexpectedly low level in Taiwan since 2021, but RSV infections strongly rebounded in late 2022 after the relaxation of public measures and restrictions. New introduced RSV-BA9 is responsible for this epidemic and is concomitantly associated with the resurgence of PIV3.

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Respiratory viruses

RSV AND INFLUENZA VIRUS CIRCULATION IN NON-HOSPEDALIZED CHILDREN DURING THE WINTER 2022-2023

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BACKGROUND-AIM

The aim of this project is to prospectively study the trends of some of the most frequent respiratory infections in children (RSV, Influenza [Flu] and SARS-CoV-2) in the community thanks to the development of a laboratory surveillance system based on a network of family pediatricians, in order to identify the circulation of these viruses and any clinical parameters of definition of the severity of this infections.

METHODS

The project involves the enrollment of children aged δ 5 years (yrs) with ARI who present in the outpatient clinics of the pediatricians participating in the programme. All patients enrolled by the network's pediatricians are given a nasopharyngeal swab. The swab is sent to the laboratory of Virology of Bambino Gesù Children Hospital in Rome and tested with an all-in-one RT-PCR multiplex molecular assay for the search of 4 targets: RSV/SARS-CoV-2/Flu-A/B.

RESULTS

To date, 122 samples related to 122 patients have been taken. Of these, 2/122 were excluded because they did not meet age requirements. Of the 120 children (60 F and 60 M, median age 1.10 yrs, IQR 0.62- 2.05), 67.5% (N=81) were positive for at least one target and 32.5% (N=39) were negative for all 4. In detail, the results showed positivity to RSV by 57.5% (N=69), to Flu-A by 8.3% (N=10), to Flu-B and to SARS-CoV-2 by 3.3% (N=4). Co-infections were detected in 6 patients: 4 RSV+FLU-A and 2 RSV+SARS-CoV-2. We then evaluated the CT of the mainly found viruses and obtained a mean CT for RSV of 24.06 and for Flu-A of 27.59. The collected data show a significant clinical impact of ARIs in the primary care setting. RSV was the predominant pathogen in the cohort under review as already shown in the pre-pandemic era and was shown to be the leading cause of respiratory illness in children <1 yrs of age.

CONCLUSIONS

In the season under review 65.8% of the patients referred to the pediatrician tested positive for RSV and/or Flu-A showing how these are the predominant viruses in the community during the epidemic season. The illness reflects moderate to severe disease with symptoms such as shortness of breath (76%) and wheezing (10%). As described in the literature, our data show a lower mean CT for RSV than the other viruses evaluated, likely indicating the severity of the infection and the need to seek medical attention.

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Respiratory viruses

SARS-COV-2 PCR POSITIVITY RATE AND VARIANTS DISTRIBUTION AMONG PATIENTS IN SOUTH CROATIA, 3-YEAR PANDEMIC PERIOD ANALYSIS

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BACKGROUND-AIM

In this analysis, we wanted to summarize data on the frequency of positive results for SARS-CoV-2 and variants distribution in South Croatia for the 3-year pandemic period.

METHODS

At the Teaching Public Health Institute of Split-Dalmatia County (SDC), during a three-year period (April 1, 2020 to March 31, 2023) we tested 473,467 patients for SARS-CoV-2 virus by real time PCR. Forward, in the period from June, 2021 to February, 2023, we sent a part of the positive results (2,095 samples) for sequencing to the Croatian Institute of Public Health Zagreb, in order to monitor the appearances of different variants during the pandemic period in South Croatia.

RESULTS

Out of the total number of 473,467 tested samples for SARS-CoV-2 virus, 159,448 (33.7%) of them were positive. The positivity rates ranged from 2.2% to 75.4%, through months in 3-year period. The highest positivity rates were detected with the appearance of variants in SDC, especially Omicron variant when the positivity rate reached 75.4% of tested patients. At the beginning of the sequencing period (June, 2021) Alpha variant was replaced by Delta variant. Then, in December 2021, Delta variant was rapidly replaced by Omicron variant, which was present until the end of the pandemic.

CONCLUSIONS

SARS-CoV-2 positivity rate in Split-Dalmatia County was most related to the appearance of new variants, with the Omicron variant being the most frequently detected.

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Respiratory viruses

SEVERE INFLUENZA DURING THE 2022/23 SARS-COV-2 PANDEMIC SEASON. CO-CIRCULATION OF SEVERE A/H3N2, A/H1N1PDM09 AND B/VICTORIA CASES IN 2023.

B. Mengual-Chuliá [7], L. Cano [6], A. Mira-Iglesias [4], S. García-Esteban [6], C. Gomis [6], R. Garrido [6], J. Pons [1], N. Jiménez-Hernandez [2], A. Orrico [4], J. Díez-Domingo [4], F.X. López-Labrador [5], .. Valencia Hospital Surveillance Network For The Study Of Influenza And Other Respiratory Viruses [3]

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BACKGROUND-AIM

We analyze the circulation of influenza viruses in emergency admissions for influenza-like illness (ILI) and severe acute respiratory infection (SARI) in the Valencia Region, Spain, during the last 2022/23 SARS-CoV-2 pandemic season.

METHODS

Prospective active-surveillance hospital-based test-negative design study on respiratory infections. ILI/SARI cases were screened in 4 tertiary-care hospitals (22% of the inhabitants), from Sep. 2022 to Apr. 2023. Swabs were tested by RT-PCR. Whole genome (WG) of influenza-positive isolates was sequenced and phylogenetic analyses were performed.

RESULTS

During the 2022/23 study period, 218 Influenza positive cases were detected. Almost half of these cases took place in late summer-autumn (n=105), reaching the peak of infection in late autumn. Interestingly, H3N2, H1N1 and B/Victoria coexisted (79%, 38%, and 11% of the positive cases, respectively). H3N2 was present from the beginning of the season to late-Feb. 2023, with a peak in Dec. 2022. H1N1 appeared in the early-autumn, with a steady number of infections throughout the season, and was still present in SARI in Apr. 2023. B/Victoria appeared in early-Feb. 2023, reaching its peak in early-March, and disappearing three weeks later.
Ninety-four WG sequences were obtained -H3N2 (n=58), H1N1 (n=26) and B/Victoria (n=10)-. All H3N2 sequences belong to different subgroups attributed to subclade 3C.2a1b.2a.2, represented by A/Darwin/9/2021. The H1N1 viruses belong to two subgroups of the clade 6B.1A.5a.2, represented by A/Victoria/2570/2019 and A/Sydney/5/2021. All B/Victoria-lineage viruses belong to subgroup V1A.3a.2, represented by B/Austria/1359417/2021.

CONCLUSIONS

After the SARS-CoV-2 pandemic, severe influenza cases have experienced an increase in the 2022/23 season, with incidences comparable to the 2018/19 SARS-CoV2 pre-pandemic season. However, this influenza season began in late summer and peaked in late autumn 2022. Like in 2021/22 season, H3N2 dominated in 2022/23. This subtype coexisted with H1N1 until the late-Feb. 2023 when H3N2 was replaced by H1N1. In addition, B/Victoria presented a small wave covering Feb.-March 2023. All the three viruses belong to clades represented by the recommended vaccine viruses for the 2022/23 northern hemisphere and 2023 southern hemisphere influenza seasons.

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Respiratory viruses

SEVERE SARS-COV-2 CASES IN 2022/23 SEASON, VALENCIA HOSPITAL SURVEILLANCE NETWORK FOR THE STUDY OF INFLUENZA AND OTHER RESPIRATORY VIRUSES (VAHNSI), SPAIN.

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BACKGROUND-AIM

For purposes of vaccine efficacy studies and to monitor for variant evolution in severe cases and antigen stability, SARS-CoV-2 cases were characterized in emergency admissions for influenza-like illness (ILI) and severe acute respiratory infection (SARI) in the Valencia Region, Spain, in the 2022/23 season.

METHODS

Prospective active-surveillance hospital-based test-negative design study on respiratory infections. ILI/SARI, cases were screened in 4 tertiary-care hospitals serving 22% of the 4,860,874 inhabitants. Swabs were tested by RT-PCR and whole genome sequencing; phylogenetic analysis and variant characterization was performed in SARS-CoV-2 positive isolates.

RESULTS

From September 2022 to March 2023, 2375 patients were admitted with ILI/SARI. Half of them (57.4%) tested positive for SARS-CoV2. From 06/2022 to 10/2022 all detected variants were omicron, with BA.5 predominating. In 11/2022 BA.5 decreased sharply and omicron BQ.1 represented 48.3% of the positive cases. By 1/2023 most omicron variants had decreased and omicron XBB variants emerged, being the XBB.1.5, the most prevalent at the present date (May 2023).

CONCLUSIONS

In our area, all the infections occurred in 2022/23 season, were due to omicron variants, predominately caused by BA.5, BQ.1, and now by XBB. Omicron continues to be dominant, alternating different variants with slight antigenic differences. It remains to be determined which updated antigens should be included in future boosters.

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Respiratory viruses

THE EMERGENCE, SPREAD, AND DOMINANCE OF THE NOVEL HUMAN METAPNEUMOVIRUS VARIANTS FROM 2014 TO 2022.

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BACKGROUND-AIM

Human metapneumovirus (HMPV) is an important aetiologic agent of respiratory tract infection (RTI), and is subclassified into HMPV-A and HMPV-B. G protein is one of the major envelope glycoproteins, and novel variants carrying two duplications of 180 and 111 nucleotides within HMPV-A G protein have been described.

METHODS

Respiratory specimens from patients with RTI suspicion at Hospital Universitari Vall d'Hebron (Barcelona, Spain) were collected from October 2014 to March 2022 for laboratory-confirmation of respiratory viruses. Partial G gene from all detected HMPV was sequenced for molecular characterisations with MEGA v6.0 (until week 13/2022).

RESULTS

A total of 90,395 specimens were collected, of which 2,051 samples (2.3%) were HMPV laboratory-confirmed. The prevalence and seasonality of HMPV remained similar in most seasons, except on 2017-2018 season (3.6% prevalence), and during the SARS-CoV-2 pandemic in 2020-2022, when HMPV's epidemic was interrupted, and later, changed the usual spring seasonality to a double peak in summer and winter 2021. HMPV-A and HMPV-B co-circulated with a cyclic predominance until 2020-2022, when HMPV-A variants upsurged, presenting a vast dominance over HMPV-B. The variants carrying these long nucleotide duplications emerged during the first two seasons, increasing in prevalence until the complete replacement of previous HMPV-A lineages. The variant carrying the 111-nucleotide duplication accounted for the 98.5% of HMPV-A variants in 2021-2022.

CONCLUSIONS

HMPV is related to a significant morbidity throughout all the study period. This virus usually presented its epidemic peak in late winter and early spring in pre-pandemic seasons. However, HMPV circulation pattern changed after SARS-CoV-2 pandemic. The common shifts in predominance between HMPV-A and -B drastically changed to a vast dominance of $A2c_{111dup}$ viruses, probably due to a more efficient immune evasion mechanism. The emergence and spread of variants carrying novel genetic features can impact on their prevalence and seasonality highlighting the importance of genomic surveillance of all respiratory viruses.

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Respiratory viruses

THE IMPACT OF THE INCIDENTAL FINDING OF RSV/INFLUENZA IN MOTHERS OF CHILDREN HOSPITALIZED IN THE NICU

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BACKGROUND-AIM

The year 2020 will go down in history as the year of the SARS-CoV-2 pandemic. During this pandemic, there was a temporary shortage of diagnostic tools to selectively detect SARS-CoV-2 through molecular testing. This forced healthcare providers to use multiplex testing in order to timely provide SARS-CoV-2 results. These tests are capable of detecting other respiratory viruses such as respiratory syncytial virus (RSV) and influenza in addition to SARS-CoV-2. This caused the accidental finding of RSV and Influenza in asymptomatic patients in a number of cases. If this involved a woman who was admitted postnatally to maternity and whose baby was admitted to the neonatal intensive care unit (NICU), this could potentially pose an infection risk to the baby.

METHODS

This study retrospectively looks at how many of Sars-CoV-2/Influenza/RSV GeneXpert tests reported an incidental finding of influenza or RSV. The data are collected based on laboratory information system (LIS) GLIMS used in tertiary Ghent University hospital (UZ Gent) in Belgium from December 2020 till April 2021 and in November and December 2021.

RESULTS

Over the period 2020-2021, 1820 and 17,718 nasopharyngeal samples were tested, respectively within context of multiplex use for SARS-CoV-2 indications. In December 2021, 4 samples positive for influenza A were found. None of the positive cases had been admitted to the maternity service. During 2021, 39 samples positive for RSV were detected. Of those 39 positive RSV samples, 2 were reported in women admitted to the maternity service. However, the babies of both women were not admitted to NICU.
A total of 43 incidental findings of Influenza or RSV on 17,718 samples tested were detected at UZ Gent in 2021 when using the Sars-Cov-2/influenza/RSV GeneXpert test. This entails 0,24% of the total samples tested with multiplex. Only 2 of those 43 incidental findings were in women admitted at maternity.

CONCLUSIONS

There are incidental finding of RSV/Influenza observed using the Sars-CoV-2/Influenza/RSV GeneXpert test for SARS-CooV-2 related indications. Even though incidental findings are not very common, they deserve attention also regarding ethical considerations in the context of potential health risk to the baby.

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Respiratory viruses

T-TRACK® SARS-COV-2, A NOVEL RT-QPCR-BASED WHOLE-BLOOD ASSAY FOR DETECTION OF SARS-COV-2 SPECIFIC T CELLS

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BACKGROUND-AIM

T cell immunity against SARS-CoV-2 plays a central role in the control of the virus. In this study, we evaluated the performance of the novel CE-marked T-Track® SARS CoV 2, a quantitative reverse transcription-polymerase chain reaction (RT-qPCR) test which relies on the combined evaluation of IFNG and CXCL10 mRNA levels, in participants with and without a history of SARS-CoV-2.

METHODS

Samples have been investigated using T-Track® SARS CoV 2, a quantitative reverse transcription-polymerase chain reaction (RT-qPCR) test which relies on the combined evaluation of IFNG and CXCL10 mRNA levels.

RESULTS

For 62 convalescent donors, 100% responded to the S1 protein while 88.7% showed a NP response. For 68 naïve donors, 4.4% responded to the S1 protein while 19.1% showed NP reactivity. Convalescent donors < 50 and ε 50 years of age demonstrated a 100% S1 reactivity and an 89.1% and 87.5% NP reactivity, respectively. T-Track® SARS-CoV-2 and serology test recomLine SARS-CoV-2 measurements according to time after SARS-CoV-2 diagnosis and immunization (by COVID 19 vaccination or SARS CoV 2 infection) showed comparable data, and still 66.7% S1 reactive samples of vaccinated and convalescent donors beyond 36 weeks.

CONCLUSIONS

Our results demonstrate a very good performance of the T-Track® SARS-CoV-2 molecular assay over time and may be suitable for COVID 19 vaccinations trials and cross-reactivity studies.

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Respiratory viruses

TWO YEAR FOLLOW UP OF RESPIRATORY VIRUSES IN WASTEWATER IN COMPARISON TO CLINICAL SAMPLES IN LEUVEN, BELGIUM

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BACKGROUND-AIM

Detailed information on the spread of respiratory viruses in the community is crucial to gain better understanding of the burden of respiratory infections on society. We explored the possibility to use wastewater sampling to monitor the circulation of respiratory pathogens at population level.

METHODS

Wastewater samples were collected from a large regional wastewater treatment plant in Leuven from January 2021 to December 2022, and were tested with an in-house respiratory panel for simultaneous detection of 29 respiratory pathogens (22 viruses and 7 bacteria/fungi), developed at the University Hospitals Leuven (UZ Leuven) diagnostic laboratory for detection of respiratory infections in clinical samples. A comparison was made to the number of positive samples reported over the same period by UZ Leuven, which drains most patients from the wider region around the city of Leuven, in patients with (mostly severe) respiratory infections.

RESULTS

We were able to detect all respiratory viruses included in the panel (influenza A and B, RSV, human metapneumovirus (HMPV), parainfluenza viruses (PIV) 1-4, adenovirus , bocavirus, enterovirus/rhinovirus (EV/RV), EV-D68, parechovirus, human coronaviruses (HCoV)-NL63, -229E, -OC43, -HKU-1 and -SARS, cytomegalovirus and herpes simplex virus -1 and -2), except for HCoV MERS of which there were no cases reported in Belgium during the two year study period. The fluctuations in community circulation of influenza A and B, RSV, HMPV and PIV 1-4 as deducted from the number of positive clinical samples and/or from data reported by sentinel labs in Belgium (when available) were in agreement with their detection in wastewater samples. EV/RV were continuously present, both in wastewater and in respiratory samples. An upsurge of EV-D68 infections in Europe in September 2021 was clearly reflected in the wastewater samples, with wastewater detection preceding reported cases. The wastewater pretreatment that was used, optimized for viral enrichment, was as such not suited for the surveillance of bacteria and fungi.

CONCLUSIONS

Community circulation levels of typical seasonal respiratory viruses were well reflected in wastewater samples, indicating that wastewater-based epidemiology can be a valuable tool in the surveillance and management of respiratory infections.

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Respiratory viruses

UNDERREPORTING AND MISCLASSIFICATION OF RSV-CODED HOSPITAL ADMISSIONS IN DENMARK

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BACKGROUND-AIM

Low awareness of respiratory syncytial virus (RSV) infections among adults has led to underdiagnosis, misclassification, underreporting in patient records, and lack of routine testing for RSV. The Danish national health registries provide an opportunity to identify all patients hospitalized with an RTI diagnosis based on ICD-10 codes and/or laboratory-confirmed RSV-tests on an individual level. The aim of this study was to assess the underreporting and misclassification of RSV infections among adults hospitalized with a respiratory tract infection (RTI)-coded hospitalization.

METHODS

This study is an observational cohort study of RSV-associated hospitalizations among Danish adults (≥18 years old) based on national healthcare registries conducted in the period of week 40, 2015 to week 40 2018. Data were extracted from The Danish National Patient Registry (DNPR) and The Danish Microbiology Database (MiBa) and linked via a unique personal identification number. We identified RSV-positive hospitalizations by linking RTI-coded hospitalizations with a positive RSV test within ±30 days of admission.

RESULTS

Using hospital admission registries, we identified 440 RSV-coded hospitalizations, of whom 420 (95%) had a positive RSV-test registered. By linking patients with RTI-coded hospital admissions to a positive RSV-test result, we found additional 570 episodes of RSV-positive hospitalizations without an RSV-coded diagnosis registered. With that, we argue, that though the specificity of the RSV-coded hospitalizations in the DNPR is high, the sensitivity is not ideal. The study showed that the reliability when using hospitalization diagnosis data to estimate the burden of RSV among adults can be questioned, even in a country like Denmark with complete nationwide healthcare data.
Studies based on healthcare data can be useful to identify general trends and observe seasonality, but to estimate the true burden of RSV among adults, investigating the excess hospitalization by using regression models or comprehensive prospective surveillance can be recommended.

CONCLUSIONS

Our study showed that RSV is underreported and underdiagnosed among Danish adults using national register data to explore the disease burden. Our results underscore the need for more attention given to the RSV burden among adults.

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Respiratory viruses

UNRAVELING THE BURDEN OF HUMAN BOCAVIRUS 1 PEDIATRIC RESPIRATORY INFECTIONS: RESULTS FROM AN 11-YEAR HOSPITAL STUDY

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BACKGROUND-AIM

Human Bocavirus 1 (HBoV1) is a small DNA virus that was first identified in children with respiratory tract infections (RTI). It has since been frequently detected with other respiratory viruses, and in children without RTI, making the burden of true HBoV1 RTIs challenging to assess. This study aimed to assess HBoV1 prevalence, codetections, seasonality, and hospitalization rates, and compare with respiratory syncytial virus (RSV) using HBoV1-mRNA PCR as a marker for an active, replicating virus.

METHODS

During an 11-year period, 4,879 children who were admitted to the hospital with RTIs were enrolled in the study. Nasopharyngeal aspirates (NPAs) were collected from all children and tested for HBoV1-DNA, HBoV1-mRNA, RSV, and 19 other respiratory pathogens.

RESULTS

HBoV1-DNA was detected in 6.2% of NPAs, making it the eighth most detected virus, and HBoV1-mRNA was detected in 2.7%. Viral co-detections were present in 74%. HBoV1-mRNA had a modest seasonal variation, peaking in autumn and winter. HBoV1-mRNA detection was more likely in the case of high HBoV1-DNA load (>106 copies/ml) and single HBoV1-DNA detection (OR 3.9, 95% CI 1.7-8.9), but less likely in samples with codetections of severe viruses such as RSV (OR 0.34, 95% CI 0.19-0.61). Children with a positive HBoV1-mRNA test were more likely to acquire a lower RTI diagnosis than those with a negative test. The mean yearly rate of hospitalizations >24 hours with lower RTI and HBoV1-mRNA was 0.70 per 1000 children <5 years old, with the highest rate in the 12-23 months age group. For RSV 8.7 per 1000 children <5 years old were hospitalized with lower RTI, and most of the children were <12 months old.

CONCLUSIONS

True HBoV1 RTI is most likely when the HBoV1-DNA load is high and when HBoV1-DNA is detected alone. HBoV1-mRNA exhibits a modest seasonal variation, with increased detection rates during autumn and winter. HBoV1 is a relatively infrequent cause of lower RTI in children, with a hospitalization rate 12 times lower than RSV.

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Respiratory viruses

VIRAL RESPIRATORY INFECTIONS IN NORTHERN GREECE, 2021-2023

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BACKGROUND-AIM

Numerous viruses cause frespiratory tract infections. Following relaxation of protective measures against the risk of SARS-CoV-2 spread, there has been an increase in the incidence of viral respiratory infections. Aim of the study was to identify the viruses (other than SARS-CoV-2 and influenza viruses) responsible for respiratory infections during 2021-2023.

METHODS

We tested 229 respiratory samples sent to the laboratory during January 2021 up to present, using two multiplex PCR assays: BioFire FilmArray Pneumonia Panel PCR (BioMeriux) applied directly on clinical samples, and RespiFinder (PathoFinder) following extraction of the genetic material. Conventional PCRs for genotyping combined with Sanger sequencing were applied on the positive samples for respiratory syncytial virus (RSV) and adenoviruses. Next generation sequencing (NGS) was applied on four selected positive samples using Oxford Nanopore platform. Barcoded sequencing libraries were prepared from 1000 ng DNA using the Ligation Sequencing kit SQK-LSK109. The library was loaded on an R9.4 flow cell and sequenced on MinION Mk1C device within 24h. Map to reference sequence was performed using Geneious Prime. Sequences were aligned with sequences available at NCBI using MAFFT v.7. Phylogenetic analysis was performed with MEGA11.

RESULTS

RSV was detected in 38 samples (16.59%), rhino/enteroviruses in 14 (6.11%), adenovirus in 9 (3.93%), bocavirus in 4 (1.75%) and parainfluenza virus (PIV)-4 in 3 samples (1.31%). Five co-infections were observed (1 RSV-A with bocavirus, 2 RSV-B with bocavirus, 1 RSV-A with HPIV-4 and 1 rhinovirus/enterovirus with adenovirus). The RSV-A stains belonged to the ON1 genotype and the RSV-B to the BA genotype. De-novo NGS identified adenovirus B3, metapneumonovirus B1 and RSV-B genotype BA, and phylogenetic trees were constructed.

CONCLUSIONS

Excluding SARS-CoV-2 and influenza viruses, the most frequently detected virus during the study was RSV-B genotype BA, followed by four additional viruses. Syndromic approach provides useful data on the epidemiology of respiratory infections, especially when combined by further genetic characterization of the viral strains.
The study was funded by Horizon 2020 VEO (874735).

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Viral infections in pregnancy

CYTOMEGALOVIRUS SEROPREVALENCE AMONG WOMEN OF CHILDBEARING AGE IN TURKEY IN A EIGHT-YEAR PERIOD

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BACKGROUND-AIM

Congenital CMV (cCMV) infection, the leading cause of nongenetic sensorineural hearing loss, is common worldwide. cCMV rate is proportional to the CMV seroprevalence of the population. Following up on the seroprevalence of CMV among women of childbearing age is crucial to determine whether screening pregnant women and newborns is necessary and to implement interventions for preventing the transmission of the infection.

METHODS

CMV-specific IgG and IgM were investigated among women of reproductive age in a eight-year period using the chemiluminescence immunoassay method. The igG avidity test was conducted using an ELISA method

RESULTS

21,783 women were included. The mean age of the women was 31.3±5.7 years. Of all women, 20,944 (96.1%) were IgG seropositive but IgM seronegative. IgG seroprevalence was found to be 94.1%, 96.9%, 94.1%, 96.7%, 96.5%, 97.3%, 98.2%, and 96.4% during the years of 2015-2022, respectively. Only three individuals were IgM seropositive but IgG seronegative. IgG avidity was investigated in 309 of 486 women who were seropositive for both IgM and IgG, and the avidity index was found to be high for all.

CONCLUSIONS

High seroprevalence of CMV among women in Turkey indicates the higher possibility of the development of cCMV infection via reactivation or reinfection rather than primary infection.
Screening strategies for newborn and interventions for increasing the awareness of CMV infections are more important than screening pregnant women in Turkey. Seroprevalence should be followed up in well-planned studies rather than routine screening.

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DEVELOPMENT OF MULTIPLEX POLYMERASE CHAIN REACTION (MPCR) FOR HCMV GLYCOPROTEIN GENOTYPING.

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BACKGROUND-AIM

Background: Human Cytomegalovirus (HCMV) is the most common congenital viral infection affecting 0.2-2.2% of all live births. Although most infected babies are asymptomatic, congenital HCMV is a major cause of sensorineural hearing loss and birth defects globally. The virus carries 6 major glycoproteins within three complexes; gC-I (gB); gC-II (gM/gN); and gC-III (gH/gL/gO or gH/gL/UL128-131). These glycoproteins are polymorphic, producing distinct genotypes and some are known to elicit neutralizing antibodies which makes them of interest for correlation with severity and outcome of disease. However, previous studies linking particular individual genotypes with disease outcomes show contradictory results.

METHODS

Methods: A novel multiplex polymerase chain reaction (mPCR) targeting genes coding for the HCMV essential glycoproteins (gB, gM, gN, gH, gL, and gO) was developed and applied to five well-characterised laboratory strains of HCMV. The amplicons were subjected to restriction fragment length polymorphism using appropriate restriction enzymes and results were validated by direct Sanger sequencing.

RESULTS

Results: All glycoproteins from each strain of HCMV were successfully amplified using the multiplex PCR and expected genotypes were confirmed by restriction digestion and sequencing.

CONCLUSIONS

The mPCR can successfully and simultaneously detect the major glycoprotein genotypes from HCMV laboratory strains. This assay may be useful in elucidating any role for glycoprotein genotypes in congenital and other CMV diseases.

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EVALUATION OF THE PERFORMANCE OF THE ALINITY M CMV ASSAY FOR THE DETECTION OF CONGENITAL CMV INFECTION IN AMNIOTIC FLUIDS AND SALIVA

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BACKGROUND-AIM

PCR in amniotic fluid (AF) retrieved by amniocentesis is the gold standard for the diagnosis of CMV fetal infection. Neonatal diagnosis relies on CMV PCR in urine or saliva collected in the first 21 days of life. The aim of this study was to compare the performance of the Alinity™ m CMV assay for prenatal and neonatal detection of congenital CMV infection in a set of retrospective or prospective clinical samples (AFs and saliva (off-label sample types)) with confirmed or excluded CMV infection using the laboratory CMV PCR assay (TOR) as reference.

METHODS

Samples: 76 AFs stored at -80°C; 218 saliva in Amies transport medium (Copan) collected at birth (49 samples stored at -80°C and 169 fresh samples). TOR was a 2-step method with DNA extraction by an Emag extractor (BioMérieux) followed by amplification and quantification with the real-time PCR assay CMV R-GENE™ (BioMérieux). Its limit of detection (LOD) was 116 IU/ml in AF and 174 IU/ml in saliva. Alinity m CMV (Abbott) is a real-time PCR assay fully automated on the Alinity m instrument with an LOD of 30 IU/mL in plasma.

RESULTS

Concordance between the two assays was 96.1% and 92.2% in AF and saliva, respectively. 1 weak positive AF with Alinity m CMV (31 IU/ml) was negative with TOR but the neonate was found positive. 2 AFs with low viral loads with TOR (34 and 514 IU/ml) were negative with Alinity m CMV; these two samples were TOR negative at retest. 17 saliva samples were positive with Alinity m CMV (low viral loads δ 2.14 log IU/ml) and negative with TOR. Correlation between viral load values was high (R2=0.91 and 0.77 for AF and saliva, respectively). Mean bias in AF and saliva was 0.40 and 1.02 log IU/ml (TOR-Alinity).

CONCLUSIONS

Alinity m CMV showed good performance compared to a reference PCR. Samples with low viral loads were more frequent with Alinity m CMV than with TOR due to a lower LOD. In 1 AF, this indicated a positive neonate. Low viral loads in saliva may be due to contamination of samples by maternal CMV DNA coming from the birth canal or breast milk and should be confirmed in a second sample. Though off-label, Alinity m CMV showed clinical utility in rapid and fully automated detection of prenatal and neonatal congenital CMV infection.

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EVALUATION OF THE SIMPLEXA™ CONGENITAL CMV DIRECT ASSAY ON URINE SAMPLES

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BACKGROUND-AIM

CMV is the most common congenital infection worldwide and is a major cause of sensorineural hearing loss (SNHL) and developmental delay. Up to 90% of infants with congenital CMV (cCMV) infection are asymptomatic at birth, making the diagnosis challenging. Testing neonatal saliva and/or urine before 21 days of life is part of postnatal diagnosis to confirm cCMV. Currently, there are limited CE-marked molecular qualitative assays specifically for neonatal congenital CMV detection using saliva swabs and/or urine as specimen types. The DiaSorin Molecular Simplexa® Congenital CMV Direct assay allows the qualitative detection of CMV DNA in a sample-to-answer format that does not require nucleic acid extraction and is currently cleared for saliva swabs and urine specimens. The Simplexa Congenital CMV Direct kit for identifying neonatal congenital CMV infections in urine was assessed in this study.

METHODS

Clinical performance was assessed using 42 collected urine specimens. The Simplexa Congenital CMV Direct kit was directly compared to the Q-PCR CMV R-GENE® kit (BioMérieux). The percent agreement and the turnaround time were evaluated.

RESULTS

Compared to the CMV R-GENE® kit, the Simplexa assay showed a total percent agreement of 97.6% (95% CI,0.87 to 0.99), with a | statistic of 0.90 (95% CI, 0.70 to 1.0). Overall, a positive percent agreement of 97.3% (95% CI, 0.86 to 0.99) was observed. The assay had 100% negative percent agreement. An overall discordance rate of 2.4% was found between the two assays. One specimen was not detected for CMV in Simplexa that the CMV R-GENE® kit did detect. The overall turnaround time for the Simplexa assay was 90 minutes, while the R-GENE® kit took 180 minutes.

CONCLUSIONS

These findings indicate that the Simplexa Congenital CMV assay can reliably detect CMV in urine, is easy to use and does not require nucleic acid extraction, which provides a faster turnaround time and less hands-on time.

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INFLUENZA VIRUS INFECTION IN PREGNANT WOMEN IN TOULOUSE UNIVERSITY HOSPITAL FROM 2017 TO 2020: CLINICAL IMPACTS IN MOTHERS AND CHILDREN, TREATMENT AND VACCINE DATA

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BACKGROUND-AIM

Influenza virus infection can be severe especially among people suffering from co-morbidities, the elderly and the pregnant women. We describe the clinical presentation of antenatal influenza, the risk factors among Flu hospitalized pregnant women during 3 winter seasons from 2017 to 2020 and the newborns perinatal outcomes.

METHODS

Clinical data, medical imagery and biological data were collected from related softwares. The odds ratios (OR) for all variables were calculated by univariate and multivariate logistic regression. Statistical significance was set at $p<0.05$.

RESULTS

This retrospective study shows that pregnant women were more frequently hospitalized when infected during the third trimester of pregnancy compared to the first and second ones.
We show no significant difference in the occurrence of preterm delivery or maternal complications whether influenza occurs in the first, second or third trimester of pregnancy.
Our data highlight a higher incidence of ceasarians in comparison with French National data ($p<0.05$).
We observed a low coverage of influenza vaccination among pregnant women in concordance with National data.

CONCLUSIONS

Influenza infection is responsible for hospitalization especially during the third trimester of pregnancy but without pejorative evolution.
Although vaccination is currently one of the most effective means of prophylaxis, it is not widely used. This despite the entire cost covering by French Health authorities for people at risk of severe forms of the disease including pregnant women. Such a policy must be reinforced and well explained.

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THE USE OF POST-EXPOSURE PROPHYLAXIS FOR VZV IN PREGNANT WOMEN IN LOTHIAN A COMPARISON BETWEEN JANUARY 2016-JULY 2018 AND JANUARY 2019 – MARCH 2023

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BACKGROUND-AIM

Primary infection with Varicella-Zoster Virus (VZV) is usually self limiting but can be complicated in those who are immunosuppressed, neonates and pregnant women. For those at risk of complicated infection, post-exposure prophylaxis (PEP) is available to ameliorate potential infection. This comparative audit focuses on the use of PEP in pregnant women at time points where the guidance on PEP differed.

METHODS

A search of VZV IgG negative results was conducted in women of child bearing age between January 2016 and July 2018; and January 2019 to March 2023. During these periods guidance was updated in August 2018 (acyclovir >20 weeks from consider to recommend), June 2019 (preferential use of acyclovir >20 weeks) and April 2022 (all pregnant woman to receive acyclovir). This data was interrogated to assess whether the women had contact with maternity services, and whether post-exposure prophylaxis was advised.

RESULTS

62 women presenting to maternity services were identified as VZV IgG negative during audit period one and 56 during audit period two. Of these, 11 of the 62 (17.7%) and 12 of the 56 (21.4%) were deemed to be inappropriately tested. 2 of the 62 (3.2%) were deemed to be inappropriate advice. No cases in audit period two were advised inappropriately. During audit period one 33 were advised to have VZIG with 20 being given. Of these 7 were potentially erroneously given. 1 was given with a history of chicken pox, 2 with IgG results of over 100IU/ml, 4 didn't have contact as defined by the Green Book. A further 2 advised to get VZIG were erroneously administered VZV vaccine. In audit period two 20 women were advised acyclovir and 13 VZIG. Of the 20, no-one was erroneously given acyclovir although one woman received VZIG. Of the 13 advised to receive VZIG 2 were not given. One due to the development of chicken pox and one as no contact had taken place

CONCLUSIONS

This study confirms the need for accurate history taking with regards to potential exposure and history of chicken pox, alongside timely antibody testing to elucidate whether pregnant patients are candidates for PEP. Clinical advice and the delivery of PEP appears to be improved since the introduction of acyclovir only guidance

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Viruses, tumors and immunocompromised hosts

BK VIRUS INFECTION IN PATIENTS AFTER ALLOGENEIC HAEMATOPOIETIC STEM CELLS TRANSPLANTATION

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BACKGROUND-AIM

BK polyomavirus (BKV) is a double-stranded DNA virus, often isolated post renal transplantation. Primary infection occurs in childhood, after which the virus persists in renal tubular cells and uroepithelium. Reactivation of latent infection in immunosuppressed patients, particularly after allogeneic hematopoietic stem cell transplantation (HSCT) and renal transplantation leading to hemorrhagic cystitis and polyomavirus associated nephropathy causes increased morbidity and complications in transplant patients. In this study, a cohort of patients after allogeneic HSCT with haemorrhagic cystitis was tested for BKV and the relationship of infection with immunosuppressive therapy, development of complications and recovery was evaluated.

METHODS

A cohort of 397 patients after allogeneic HSCT in University Hospital Hradec Kralove was followed during the period between 2011-2022. Detection of BKV DNA in blood and urine in patients with a suspicion of BKV infection (with dysuria, haematuria and strong pain) was performed using in-house quantitative PCR with the primers from LT-Ag region.

RESULTS

From a total of 84 BKV positive patients (21,15 % of all HSCT in the cohort), 42 were males and 42 females, predominantly older than 60 years. The patients (64 with unrelated and 20 with related HSCT donors) were on standard immunosuppression regimen of tacrolimus/mycophenolate mofetil. In 11 patients the beginning of the BKV infection and clearance of the virus was identified from repeated samples. AML was the most frequent diagnosis in these patients. Clinical symptoms improved mostly after symptomatic therapy (hydration, analgesics) within the period of 10 to 87 days. Viral loads in urine of the patients reached 10^8 - 10^{10} copies/ml in most patients, while the copy numbers in plasma were generally below 10^2 -10^3 copies/ml. In some patients increased plasma creatinine levels were associated with BKV infection.

CONCLUSIONS

The results from this cohort showed an extended recovery period with the median of 35 days, contrary to shorter recovery periods reported in the literature. This indicates, that while the BKV infection is usually not life threatening, it can pose serious complications in HSCT recipients, which can extend their recovery.

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Viruses, tumors and immunocompromised hosts

CHARACTERISTICS AND CLINICAL COURSE OF PARAINFLUENZA VIRUS TYPE 3 INFECTIONS IN A SERIES OF 20 CASES IN A HEMATOLOGY UNIT

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BACKGROUND-AIM

Parainfluenza virus type 3 (PIV3) can be responsible for mild to severe respiratory infections and hospital epidemics. We aim to describe a series of cases in a hematology unit.

METHODS

Respiratory viruses were screened by multiplex PCR (Respiratory Panel 2.1 plus, BioMérieux) and the PIV3 load was determined for positive samples by quantitative real-time PCR. The clinical characteristics and course of the infections are described.

RESULTS

Between November 2022 and January 2023, among 134 hospitalized on the unit, 113 patients were tested and 20 were identified as PIV3 positive (17.7%). Four patients had co-infection with at least one other respiratory virus. PIV3 was identified during hospitalization in 12 patients. The other 8 cases were detected on the day of admission, 7 having previously stayed in the department during the same period and one being admitted for the first time. At the same time, the frequency of PIV3 infection in the rest of the hospital was significantly lower ($p<0.0001$).
All infected patients suffered from a hematological malignancy (including 14 myelomas). Of the clinical data collected for 18 patients, the main symptoms observed were fever (n=11.6%), cough (n=9.5%), rhinitis (n=1.6%). Four patients had polypnea (22%), nine had dyspnea (50%) of which six were oxygen dependent (33%). Over the period analyzed, a diagnosis of viral pneumonia was made in 10 cases (56%), six of which were attributed solely to PIV3 (33%), including three deaths. The observed mortality was 30% in PIV3-positive patients and 2% in PIV3-negative patients ($p=0.0003$).
The mean minimum duration of viral shedding was 21.8 days (12-34 days). The mean respiratory PIV3 load was 8.6 log10 copies/ml at the first positive sample, with no significant difference between patients with and without pneumonia.

CONCLUSIONS

This series demonstrates the epidemic potential of PIV3 in a hematology unit. Phylogenetic analyses will establish the links between the strains. PIV3 infections were associated with a high frequency of pneumonia. These results underline the interest of including PIV3 testing in the microbiological diagnosis of respiratory infections in patients with hematologic malignancies and the need to conduct therapeutic trials with molecules active against parainfluenza viruses in this population.

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Viruses, tumors and immunocompromised hosts

CLINICAL UTILITY OF CYTOMEGALOVIRUS GENOTYPIC DRUG-RESISTANCE TEST IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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BACKGROUND-AIM

Cytomegalovirus (CMV) infection represents a severe complication in hematopoietic stem cell transplant (HSCT) recipients. Prolonged exposure to anti-CMV drugs can lead to the onset of viral genome mutations associated with drug resistance (DR). This study evaluates the clinical utility of genotypic test for CMV-DR in HSCT recipients with refractory CMV infection.

METHODS

We evaluated 27 episodes of refractory CMV infection from 27 HSCT recipients, 15 males and 12 females (median age 44 years). Only 1 patient was seronegative for CMV prior to transplant (R-). UL97 and UL54 viral genes were analyzed by Sanger sequencing to assess the resistance-associated mutations (r) for valganciclovir/ganciclovir ([V]GCV), foscarnet (FOS), cidofovir (CDV), maribavir (MBV). To detect letermovir (LTV) resistance, we investigated UL56, UL89, UL51 genes.

RESULTS

CMV-DR mutations were identified in 10/27 cases (37%). In 6/10 (60%), 6 single DR mutations on UL97 gene associated with (V)GCVr (n=2 A594V, n=3 L595S, n=1 M460V) were found. Treatment was replaced by FOS (n=5) and CDV (n=1). Mutations associated to multidrug resistance (MDR) were found in 1 episode: A594P (UL97) with V715M (UL54) conferring GCVr and FOSr. As a result, therapy was modified with MBV, although the immunosuppressive therapy has not been changed. In 3/10 (30%) cases, single mutations (n=2 C325Y, n=1 R369S) in UL56 gene associated with LTVr were detected. Each patient was in off-label treatment with LTV. Therapy was switched to VGCV (n=2) and FOS (n=1). Moreover, among the 10 cases with CMV-DR, none developed a CMV-related disease. Finally, in the remaining 17/27 (63%) episodes of refractory CMV infection, no mutation associated to CMV-DR was detected by CMV-DR test. In 10/17 (59%) of these patients, a second-line treatment was avoided. Among them, 1 case developed CMV-associated chororetinitis/uveitis.

CONCLUSIONS

Results showed the key role of genotypic testing for CMV-DR in the clinical management of HSCT patients with CMV infection refractory to antiviral treatment.

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Viruses, tumors and immunocompromised hosts

CLINICAL VALIDATION OF THE NEUMODX HPV ASSAY WITH SELF-COLLECTED (CERVICO-) VAGINAL SPECIMENS

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BACKGROUND-AIM

High-risk HPV (hrHPV) DNA testing on self-collected cervico-vaginal specimens (HPV self-sampling) has similar clinical performance to clinician-taken cervical samples and helps increase cervical screening coverage by reaching former underscreened women. This study describes the clinical validation of the novel NeuMoDx HPV Assay (QIAGEN, GmbH) self-sampling workflow.

METHODS

Study design was a cross-sectional clinical equivalence study of the NeuMoDx HPV Assay self-sampling workflow compared to that of a validated reference assay (HPV-Risk Assay, Self-Screen B.V.) to assess clinical sensitivity, specificity and reproducibility. Design and criteria used to clinically-validate the novel self-sampling workflow using the Evalyn Brush (Rovers Medical Devices, NL) were adopted from the criteria used for physician-taken cervical samples. Equivalence was assessed with the non-inferiority score test. Validation covered all aspects from sample collection to result analysis. Discordant results were analysed with a second clinically-validated assay (GP5+/6+-PCR EIA Assay, Self-Screen B.V.), including full genotyping.

RESULTS

The NeuMoDx HPV Assay self-sampling workflow demonstrated a relative clinical sensitivity for CIN3+ of 1.016 compared to the reference assay (p=0.0046). Relative clinical specificity compared to the reference assay was 0.996 (p=0.0088). Intra-laboratory reproducibility over time was 94.4% (95% CI 92.5-99.6), and inter-laboratory agreement among the 3 different labs was 93.8% (95% CI 91.8-95.6; Lab 1 vs Lab 2) and 93.4% (95% CI 91.4-95.3; Lab 1 vs Lab 3).

CONCLUSIONS

The NeuMoDx HPV Assay self-sampling workflow using the Evalyn Brush met all criteria for intra-laboratory reproducibility and inter-laboratory agreement and the clinical sensitivity and specificity were shown to be non-inferior to those of the reference assay, with high success rates for testing self-samples (ε99%). Discrepant results were not related to a specific hrHPV type and were mostly due to late Ct values around the assay cut off. The NeuMoDx HPV Assay self-sampling workflow can therefore be considered clinically-validated for HPV self-sampling.

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CONTRIBUTION OF A DOSAGE OF ANTI-B19 IGG IN THE INTRAVENOUS IMMUNOGLOBULIN VIALS ?

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BACKGROUND-AIM

In immunocompromised patients, Parvovirus B19 (B19) viremia may persist and may be responsible for a chronic infection. Without specific antiviral therapy, intravenous immunoglobulin (IVIG) are the only treatment for persistent infection, as supportive therapy for immunocompromised patients. Treatment efficacy depends on the presence of neutralizing anti-B19 antibodies in the vials administered to the patients. This study aims to evaluate the qualitative and quantitative anti-B19 IgG detection in different IVIG batches.

METHODS

We first evaluated the LIAISON® Biotrin Parvovirus B19 IgG assay linearity with 6 patient sera. We performed serial twofold dilutions in PBS. With this assay, anti-B19 IgG result is positive when ratio is greater than or equal to 1.1. Then, we collected all IVIG vials dispensed by the hospital pharmacy between March 10 and 23, 2023 (all indications combined). We measured anti-B19 IgG ratios in each vial recovered after administration to patient. We performed serial twofold dilutions when the initial result was positive (above 1.1) and with a ratio higher than 25 to determine more precisely the anti-B19 level in each tested vial.

RESULTS

We first validated the linearity of the assay and determined that the LIAISON® Biotrin Parvovirus B19 IgG assay has a linear range between 1.1 and 25 ($R^2>0.99$). In total, we tested IVIG from 4 different manufacturers and from 10 batches. We detected anti-B19 IgG in similar levels in all tested IVIG vials. Median anti-B19 IgG level was 110 (min 80 – max 140).

CONCLUSIONS

The linearity of the LIAISON® Biotrin Parvovirus B19 IgG assay has been validated for ratios up to 25. All tested IVIG vials contain anti-B19 IgG without difference according to the IVIG specialty. We didn't show evidence for some variability in anti-B19 IgG levels according to the batch or the manufacturer. This study is proof of concept and should be validated in a large series to determine whether a dosage of anti-B19 IgG level before IVIG administration could be predictive of treatment efficacy in immunocompromised patients with chronic parvovirus B19 viremia.

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Viruses, tumors and immunocompromised hosts

CYTOMEGALOVIRUS (CMV) VIRAEMIA AND SEROCONVERSION IN CMV SERONEGATIVE SOLID ORGAN TRANSPLANT RECIPIENTS MANAGED WITH EARLY PRE-EMPTIVE TREATMENT

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BACKGROUND-AIM

Cytomegalovirus (CMV) seronegative solid organ transplant (SOT) recipients are at high risk of CMV infection and end-organ disease if the donor is CMV seropositive (D+R-). At the Royal Free Hospital (RFH) in London, United Kingdom, pre-emptive treatment (PET) with valganciclovir is started in D+R- renal and liver transplant patients following the first detectable CMV DNA (ε200 copies/ml) in whole blood tested by PCR for surveillance. We undertook a review to re-evaluate the efficacy of this strategy in preventing CMV disease and examine the frequency of recurrent CMV infections.

METHODS

A retrospective review of electronic patient records and the laboratory information management system was conducted for D+R- patients undergoing SOT from April 2021 to January 2023 for renal (D+R- n=25) and June 2021 to January 2023 for liver (D+R- n=27).

RESULTS

Of 27 D+R- liver transplants (17% of 154 transplants), 23 patients developed CMV viraemia within the 90-day surveillance period requiring PET. Mean time to 1st viraemia was 23 days (range 14-38), mean peak viral load 16,304 copies/ml (range 210-260,000 copies/ml), mean time to clearance 34 days. Of 15 patients followed at RFH, 13 had recurrent viraemic episodes (range 3,200-380,000 copies/ml). Of 10 patients tested 8 became CMV IgG positive. One patient had 4 recurrences, 2 post-seroconversion. No patients developed CMV end-organ disease (EOD). Of 25 D+R- renal transplants (9% of 271 transplants), 15 patients developed CMV viraemia requiring PET. Mean time to 1st viraemia was 59 days (range 15-332). The mean peak viral load was 4772 copies/ml (range 210-30,000 copies/ml), mean time to clearance 17 days. 12 patients had CMV viraemia recurrence (range 3100-1,400,000 copies/ml). 2 patients had 6 episodes; 3 post-seroconversion. Of 13 patients tested 11 seroconverted. One patient developed UL97 mutations conferring ganciclovir resistance due to poor compliance and was treated with maribavir. No patient developed CMV EOD.

CONCLUSIONS

PET is a safe and effective strategy for preventing CMV EOD in D+R- SOT. Some patients had recurrent viraemia despite IgG seroconversion. CMV-specific immunoassays could be useful to identify patients with poor T-cell responses. These patients can be targeted for future CMV vaccines to prevent CMV related morbidity

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Viruses, tumors and immunocompromised hosts

DETECTION OF ANAL HPV FROM 2018 TO 2023 IN MILAN, ITALY: AN OBSERVATIONAL RETROSPECTIVE STUDY

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BACKGROUND-AIM

Human Papillomavirus (HPV) is the most common microorganism responsible for sexually transmitted infections (STIs). HPV is an oncogenic virus that can cause anal lesions, it is also responsible for up to 90% of cases of anal cancer. More than 100 genotypes have been isolated, classified as high-risk (hr) and low-risk (lr) HPV. We evaluated the presence and distribution of HPV genotypes on anal samples of patients attending a large sexual health clinic from January 2018 to March 2023

METHODS

Anal samples were collected using the ThinPrep™ Pap Test PreservCyt™ (Hologic, USA) system. Nucleic acid extraction was performed using the STARMag (Seegene, South Korea) kit on Microlab NIMBUS (Hamilton, USA) platform. HPV genotyping was performed by means of AnyplexTM II HPV28 Detection (Seegene, South Korea) Real-Time Polymerase Chain Reaction commercial kit, which can detect 19 hrHPV and 9 lrHPV genotypes, respectively

RRESULTS

A total of 1754 anal samples, from 878 patients (94% men), were tested for HPV DNA: 1407 (80%) resulted positive for viral DNA. Both the overall number of tests and the percentage of HPV-positive slowly increased during the study period: 74% (102/138) in 2018, 80% (473/591) in 2019, 80% (185/231) in 2020, 79% (210/266) in 2021, 82%(366/444) in 2022 and 85% (71/84) in the first trimester of 2023. As expected, HPV 16 and HPV 6 were the most frequently detected hr and lr genotypes. At least one hrHPV genotype was detected in 86% (88/102), 90% (424/473), 88% (162/185), 93% (195/210), 89% (326/366) and 90% (64/71) of cases; hrHPV coinfections were detected in 57%(2018), 65% (2019), 60% (2020), 67% (2021), 57% (2022) and 52% (2023) of cases. The most relevant negative trend was registered for HPV 18 detection that declined from 14% in 2018 to 4% in 2023 while the most relevant increase was registered for HPV 33 detection that increased from 8% in 2018 to 18% in 2023

CONCLUSIONS

Although the increase in positivity rate over time may be related to many factors, such a high percentage of positivity is still significant in terms of reinforcing the necessity of a screening guidelines in both immunosuppressed or competent people as well as in terms of pointing out the possible risk factors associated with anal epithelial dysplasia progression

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FACTORS ASSOCIATED WITH THE LEVEL OF EPSTEIN-BARR VIRUS (EBV) VIRAL LOAD (VL): A PRELIMINARY STUDY

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BACKGROUND-AIM

Monitoring EBV viral load (VL) is important for the detection and management of EBV-associated infections, malignant-premalignant processes, and lymphoproliferative disorders. However, use of VL levels has limitations due to lack of clinical threshold standardization. The objective of this study was to identify possible factors associated with the levels of EBV viral load.

METHODS

The study was designed as a cross-sectional study. The data of 20045 samples accepted in virology laboratory of Medical Microbiology Department of Ege University Faculty of Medicine between January 2009 and May 2023 were analysed. Age, gender, clinical services where samples were collected, patients' diagnoses, treatments, statue of infection (acute/reactivation), intervals between the test and other laboratory parameters (acute phase reactants, serological tests, haematological tests) were considered as independent variables. The positivity of EBV DNA VL relationship with these variables were analysed. The results of the routinely used Artus EBV RG PCR Kit (Qiagen, Hilden, Germany) were used as EBV VL values.
The descriptive characteristics of the study were expressed as percentage. For the analysis of positive cases and independent variables, ANOVA, Chi-square and the Student's t test were used. The significance level is accepted as $p<0.05$.

RESULTS

83.1% of the patients were children and 54.7% were male. The average age was 8.1 years for children and 38.0 years for adults. The rate of positive results in all data is 18.5%. Significantly higher EBV DNA positivity rate was found in samples obtained from children (19.3%; $p<0.001$), females (19.9%; $p<0.001$) and organ transplant patients (26.5%; $p<0.001$). Viral load levels were similar in females (Median 2.46 Log10; Mean rank 1791) and males (Median 2.45 Log10; Mean rank 1775). Viral load was higher in adults (Median 2.67 Log10; Mean rank 1953) than in children (Median 2.42 Log10; Mean rank 1755) ($p<0.001$). Compared to the other clinics, samples collected from the organ transplant unit had a significantly higher viral load (Median 2.76 Log10; Mean rank 1990) (p 0.001).

CONCLUSIONS

Based on laboratory and clinical experience, each clinic should establish its own algorithm and standards for VL follow-up by close monitoring the data, particularly for immunosuppressed patients.

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GENETIC DIVERSITY OF VP1 REGION OF BK POLYOMAVIRUS IN RENAL TRANSPLANT RECIPIENTS IN SERBIA

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BACKGROUND-AIM

A prolonged state of immunosuppression has singled out BK virus (BKV) as a significant opportunistic pathogen in renal transplant recipients (RTR). BKV reactivation in RTR can lead to polyomavirus-associated nephropathy and consequent graft loss. Mutations in the VP1 region may alter the biological properties of the BKV and lead to selection of more aggressive forms with altered tropism and increased pathological potential. BKV is classified into four genotypes based on polymorphism within VP1 gene. This study aimed to determine the distribution of BKV genotypes and the presence of mutations in VP1 region in a group of RTR in Serbia.

METHODS

The study included 190 RTR. One blood and one urine sample were taken from each patient. BKV-DNA was detected by using the PCR. The positive PCR products were sequenced and analyzed by using bioinformatics tools.

RESULTS

34/190 (17.9%) patients had detectable BKV-DNA in either urine or blood. 13/190 patients (6.8%) were simultaneously positive for BKV in urine and blood. BKV-DNA was detected only in urine in 13/190 (6.8%) and only in blood in 8/190 (4.3%) patients. BKV-DNA was detected in 47 samples (27 urine and 20 blood samples). Among the 47 BKV isolates, the most dominant genotype was I 34/47 (78.7%), followed by genotype IV 10/47 (21.3%). Within genotype I, 3 subtypes were detected: Ia (21.6%), Ib1 (5.4%) and Ib2 (73%). In 5/13 (38.5%) patients, who were simultaneously positive in urine and blood, different BKV genotypes were detected in urine and blood. Ib2 (100%) was present in the urine of all 5 patients, while Ia (3/5; 60%) and IV (2/5; 40%) were detected in the blood. Eight different mutations in VP1 were detected in urine samples of 29.6% of patients. Five mutations (62.5%) were detected in BC-loop and 3 (37.5%) in ®-sheet. Twenty different mutations were found in the blood of 30% of patients, of which 16 (80%) were in the BC-loop and 4 (20%) in the ®-sheet.

CONCLUSIONS

The most frequent mutation was E73G. Among BKV isolates, subtype Ib2 was the most frequently represented. Most of the mutations were detected in the BC-loop, which plays a crucial role in binding to receptors on the target cells. Different isolates of BKV in urine and blood detected indicate a possible mixed infection with BKV.

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HIGH TTV SPECIES DIVERSITY AND VARIABILITY IN RENAL TRANSPLANT RECIPIENTS

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BACKGROUND-AIM

Torque Teno Virus (TTV) is a nonpathogenic and ubiquitous ssDNA virus, member of the Anelloviridae family, classified into 22 species (including more than 100 different strains).
Objective: to determine TTV species diversity in renal transplant patients and to evaluate species variability within the same host.

METHODS

Plasma samples from recipients who underwent renal transplantation at CEMIC University Hospital were prospectively evaluated for TTV viral load before and during the first year after transplantation.
TTV was detected using two assays (Home-Brew PCR and R-GENE®PCR). TTV typing was performed by whole genome sequencing. ORF-1 phylogenetic analysis was performed using the Maximum Likelihood method. Species variability was determined before and after transplantation.

RESULTS

A total of 107 patients were enrolled from November 2018 to April 2021. All patients were TTV positive at some point after transplantation. At the pre-transplant period, TTV median viral loads were 3.0 and 2.8 Log10 copies/mL with Home-Brew PCR and R-GENE®PCR, respectively. At month 3, median viral loads were 6.8 Log10 copies/mL (Home-Brew PCR) and 6.1 Log10 copies/mL (R-GENE®PCR).
A total of 30 plasma samples from 27 patients -randomly selected- were successfully typed. Overall, 16 different species were detected.
Most prevalent species were TTV3 (63%) and TTV24 (50%), followed by TTV18 (43%) and TTV13 (43%).
Median number of TTV species per patient was 4 (IQR: 2-6.3). Up to 10 TTV species were detected in one patient.
In the pre-transplant period, 12/30 (40%) samples showed the highest median number of TTV species per sample, 5 (IQR: 4-7.5). At month 3, median TTV species per sample was 3.5 (IQR: 2-7).
Variability: the post-transplant sample from three patients showed a decrease in the number of TTV species compared to the pre-transplant sample. Specifically, 2/3 had different TTV species. In addition, 1/3 patient's post-transplant sample had Torque Teno Mini Virus (TTMV).

CONCLUSIONS

High TTV species diversity was documented in renal transplant patients, especially in the pre-transplant period.
Higher TTV species diversity was not associated with a higher TTV viral load.
Total species variability within the same host was detected.

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HUMAN PAPILLOMAVIRUS DETECTION AND TYPING IN CERVICAL SAMPLES OF WOMAN

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BACKGROUND-AIM

Background: It has become important to perform HPV genotyping in the most sensitive way in order to use the HPV vaccine and to give the right treatment.

METHODS

Methods: Our study is a retrospective, single central observational study evaluating the data of the last 2 years. We describe the RT-PCR method for detection of cervical samples in a routine laboratory which tests about 2515 samples for 2 years, and were assessed using a real-time PCR. Positive samples were genotyped using kit.

RESULTS

Results: Among the 2515 samples, 1790 samples were negative and 725 were positive. Among HPV positive samples 29,7% in high risk group, with 13,3% HPV type16, 4,55% of type 18, 6,62% HPV type31, 1,2% HPV type 33, 2,62% type 35 and 1,37% HPV type58. Low risk were obtained below 30,3% 436 samples. %6,3 HPV type39, 1,9% type 45, 6,48% type51, 3,17% type52, 4,13% type 56, 2,2% type59, 2,6% type66 and 3,4% type68.

CONCLUSIONS

Conclusion: HPV-DNA real-time PCR assay allows confident HPV detection and typing in cervical samples that should facilitate the diagnosis of high rish HPV infection in patients.

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HUMAN POLYOMAVIRUSES IN PSORIASIS PATIENTS ON BIOLOGIC TREATMENT

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BACKGROUND-AIM

Biologic therapy poses a higher risk of viral infection for patients, however current knowledge of human polyomavirus infection in psoriasis patients is still limited. In this study, we evaluate the prevalence of BK polyomavirus (BKPyV), JC polyomavirus (JCPyV) and Merkel cell polyomavirus (MCPyV) in the group of psoriasis patients treated with biologics or topical therapy.

METHODS

A total of 267 patients were screened for the presence of polyomavirus DNA by qPCR and polyomavirus-specific antibodies by ELISAs. Of these, 110 (41.2%) were treated with topical therapy, 84 (31.5%) with anti-TNF-〈 therapy, 31 (11.6%) with anti-IL-12/23 therapy, and 42 (15.7%) with anti-IL-17 therapy for at least 6 months.

RESULTS

None of the sera tested were positive for BKPyV and JCPyV DNA and the presence and level of antibodies were comparable in all patients' groups. On the other hand, we found a statistically significantly higher prevalence of genital MCPyV infection in all groups of patients on biological therapy (46.4% for anti-TNF-〈, 35.5% for anti-IL-12/23, and 42.9% for anti-IL-17) compared to patients on topical therapy (24.5%). The prevalence of oral MCPyV infection was slightly higher in the groups with topical and anti-TNF-〈 therapy (31.8% and 34.5%, respectively) compared to patients on anti-IL-12/23 and anti-IL-17 therapy (19.4% and 26.1%, respectively), but without statistical significance. The seroprevalence was almost identical in all tested groups.

CONCLUSIONS

Our results indicate that biologic therapy is not associated with BKPyV and JCPyV reactivation and with the risk of associated diseases. The MCPyV persistence and shedding in the genital location might be enhanced by decreasing of inflammatory cytokine levels and thereby immune response by biological therapy.

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LARGE-SCALE COHORT STUDY OF THE PREVALENCE OF CUTAVIRUS IN MALIGNANT AND NON-MALIGNANT TISSUES

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BACKGROUND-AIM

Cutavirus (CuV), the newest human parvovirus, has recently received attention due to its association with cutaneous T-cell lymphoma (CTCL), a group of rare skin malignancies of unknown etiology. CuV skin persistence is also linked to parapsoriasis, an inflammatory condition and precursor of CTCL; in contrast, it has been observed only sporadically in other skin diseases or healthy skin. The aim of this study is to confirm the CuV-CTCL association with a larger number of international patient cohorts, including immune deficiencies, malignancies, and diseases with T-cell infiltration.

METHODS

We searched for CuV DNA, by quantitative PCR, in a total of 84 patients (73 from Finland and 11 from the UK), of which 26 were diagnosed with CTCL, 1 with the pre-CTCL parapsoriasis, 36 with other cancers, and 15 with healthy skin. We obtained a total of 95 FFPE skin biopsy specimens: 29 CTCL, 16 melanoma, 26 benign nevi, 17 dysplastic nevi and 7 lichen planus. Fourteen of 35 patients with nevi had also a history of melanoma. Analysis of additional international samples is ongoing. Further, visualization of CuV DNA and its target cells by RNAscope in situ hybridization and immunohistochemistry will be presented.

RESULTS

CuV DNA was detected in 5/95 (5.3%) FFPE specimens: 2/29 CTCL (6.9%), 1/17 dysplastic nevus (5.9%), 1/26 benign nevus (3.8%) and 1/3 melanoma in situ. The CuV-DNA loads varied between 10^{2}-10^{7} copies/10^{6} human cells. Interestingly, the patient with a CuV-positive dysplastic nevus also had parapsoriasis and basal cell carcinoma, and the patient with a CuV-positive benign nevus had Bowen's disease. Thus, in all, 3/30 (10%) patients with CTCL or pre-CTCL harbored CuV DNA in skin, in contrast to 0/26 (0%) patients with current or past malignant melanoma. None of the healthy patients or those with lichen planus, atopic dermatitis or psoriasis, carried CuV DNA.

CONCLUSIONS

The first step towards understanding the putative role of CuV in CTCL carcinogenesis, is elucidating the prevalence of this virus in a large number of patient cohorts. This ongoing study will reveal if the CuV-CTCL association stands, and is one step closer to revealing whether CuV only thrives in cancer cells or possibly causes cancer.

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LOWER TTV VIRAL LOAD IN RENAL TRANSPLANT PATIENTS WITH SUBCLINICAL GRAFT REJECTION COMPARED TO PATIENTS WITH NO GRAFT REJECTION

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BACKGROUND-AIM

Torque Teno Virus (TTV) is a nonpathogenic, non-enveloped, small DNA virus that is highly prevalent in the general population (90%). TTV has been proposed as a surrogate marker of immunosuppression in transplant patients.
Objective: to determine the association of TTV viral load in renal transplant patients with subclinical graft rejection compared to patients with no graft rejection.

METHODS

A prospective cohort study on adult renal transplant recipients transplanted between November 2018 and April 2021 is being conducted at CEMIC University Hospital, Argentina.
TTV viral load was determined in plasma samples collected before and after renal transplantation (at 1, 3, 6, 9 and 12 months) by two PCR assays (Home-Brew and R-GENE®).
All patients were screened for graft rejection by renal biopsies ("protocol") to detect subclinical events at two predefined times (between months 3-6 and at month 12). Additional biopsies ("indication") were taken with renal dysfunction.
Association between TTV viral load and subclinical graft rejection was determined in the last sample obtained before the rejection event.

RESULTS

A total of 746 plasma samples from 107 recipients were collected during the first 15 months after transplantation. Overall, 135 renal biopsies ("protocol" and "indication") were obtained from 92 patients.
Graft rejection was documented in 28/92 (30%) recipients. All patients, regardless of their clinical outcome, remained with a functional graft.
Of them, 10/28 (36%) patients had subclinical graft rejection. T-cell mediated rejection was detected in 7/10 patients, borderline changes were detected in 2/10 patients and antibody mediated rejection was detected in 1/10 patients.
TTV viral load was significantly lower in patients with subclinical graft rejection between months 6-9 post-transplantation. TTV median viral loads were 3.1 Log10 copies/mL (Home-Brew) and 2.4 Log10 copies/mL (R-GENE®) compared to 6.1 (Home-Brew) and 6.0 (R-GENE®) from 74 patients without graft rejection (p=0.047 and p=0.017).

CONCLUSIONS

The most frequent rejection type associated with subclinical graft rejection was T-cell mediated rejection.
TTV viral load was consistently lower in patients with subclinical graft rejection, achieving statistical significance at 6-9 months after transplantation.

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PATHOGEN NON-SPECIFIC CELLULAR RESPONSE ALONG WITH CMV-SPECIFIC AND VIRAL KINETICS COULD HELP MANAGING CMV INFECTION IN SOLID ORGAN POSITIVE RECEPTORS

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BACKGROUND-AIM

Cytomegalovirus (CMV) causes morbidity in solid organ transplant (SOT) recipients. Infection is guided by viral load quantification (VL) without a cutoff for antiviral treatment. Viral replication kinetics could predict the course of infection in hematopoietic precursor transplant and CMV-scpecific celular response has been used to estimate the risk of infection in SOT. Pathogen non-specific celular response methods have recently been developed. Aim: To estimate the usefulness of viral kinetics, CMV-specific and pathogen non-specific celular response in the management of CMV infection in CMV+ recipients.

METHODS

A prospective study of 84 CMV+ SOT recipients was conducted (2018-2022), followed with preemptive therapy or valganciclovir prophylaxis strategy. Viral load (IU/mL) was monitored with RealStar CMV (Altona) up to 6 months. Infection was treated with ganciclovir or valganciclovir. Time to VLpeak from transplantation or prophylaxis ending (tmaxt), from first detected VL>150 (tmaxc), viral doubling time from VL>150 to VLpeak (DTVpm), from 1st to 2nd VL>150 (DTV, cutoff=2) and clearance time to negative VL (VCT) were determined. CMV Quantiferon and Monitor (Biorad, Germany) were performed acording to manufacturers protocol (IFN expressed in ng/mL, low, moderate or high).

RESULTS

49 patients (58.3%) replicated and 22 (44.9%) were treated with VL peaks of 9760IU/mL (1830-131000) vs 1460IU/mL (170-6570) of untreated patients ($p<0.0005$). Treated patients reached VL peak earlier than untreated, both tmaxt ($p=0.0003$) and tmaxc ($p=0.015$). VCT was lower in treated than in untreated ($p<0.0005$).DTV was higher in treated than in untreated unlike DTVpm both without significance ($p=0.17$ and $p=0.50$). Ten patients with DTV>2 (71.4%) cleared the virus spontaneously. Patiens without infection showed higher Monitor response than infected (IFN amounts and level percentages).

CONCLUSIONS

Patients with antiviral treatment had higher VL peak, reached it earlier and cleared the virus faster than untreated. Patients without treatment and DTV>2 days cleared the virus spontaneously (>70%). Differences in cellular response between infected and non infected patients were better seen in Monitor than in CMV specific response. Monitor along with Quantiferon CMV and DTV could help in managing treatment in CMV+ transplant recipients.

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PERFORMANCE EVALUATION OF ALINITY M BK VIRUS ASSAY

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BACKGROUND-AIM

BK virus is a common DNA virus of the polyomavirus family that has approximately 80% seroprevalence in healthy blood donor. Infection is generally asymptomatic in immunocompetent individuals but is the primary cause of tubulo-interstitial nephritis and ureteral stenosis in renal transplant recipients. Quantitative assessment of BKV is one of the most effective tools for supporting treatment management. Here we evaluated analytical performance of Alinity m BKV assay in plasma and urine.

METHODS

Key assay attributes, linearity, precision, and limit of detection (LOD) of the assay were evaluated in both plasma and urine, the latter stabilized in the Alinity m Transfer Buffer. Linearity was tested with BKV concentrations ranging between 1.7 to 9.0 Log IU/mL Analytical precision of Alinity m BKV was assessed by testing an 8-member plasma panel and a 9-member urine panel twice each day for 5 days on 3 instruments by 3 operators totaling a minimum of 120 replicates per panel member. Panel for testing the LOD was prepared by diluting the 1st World Health Organization (WHO) international Standard for BKV in negative plasma and urine.

RESULTS

Alinity m BKV demonstrated linearity across the quantitation range from 1.7 log IU/mL to 9 log IU/ml in both plasma and urine, for subgroups Ia, Ib and Ic, and subtypes II, III, and IV. Alinity m BKV demonstrated a within-laboratory SD of δ 0.14 Log IU/mL for plasma and δ 0.16 Log IU/mL for urine within the quantitation range. Probit analysis of the data for the least sensitive reagent lot demonstrated LOD at 18.13 IU/mL (95% CI: 11.82 to 41.99 IU/mL) in plasma and 16.97 IU/mL (95% CI: 11.42 to 39.14 IU/mL) in urine using 1st WHO BKV Standard (subgroup Ib). BKV for subgroups Ia and Ic, and subtypes II, III, and IV were detected at 50 IU/mL 100 % of the time.

CONCLUSIONS

Alinity m BKV assay delivers highly sensitive detection of diverse BKV subgroups and subtypes and accurate quantitation across a wide dynamic range in both plasma and urine specimens. The Alinity m BKV assay is performed on the fully automated Alinity m platform which provides short turnaround time testing of urgent samples and same-day reporting of test results thus shortening the time between diagnosis and treatment.

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PERFORMANCE EVALUATION OF ALINITY M CMV WHOLE BLOOD ASSAY

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BACKGROUND-AIM

Quantitation of CMV in whole blood is being used in conjunction with clinical presentation and other laboratory markers as an aid in the diagnosis and management of CMV in transplant and other immunocompromised patients. Alinity m CMV quantitative real-time PCR assay employs a dual-target design to detect highly conserved regions of the CMV genome. The objective of this study was to evaluate the clinical performance of the improved Alinity m CMV whole blood assay when compared to RealTime CMV whole blood assay on the m2000 sp/rt system used in routine clinical testing.

METHODS

Sample preparation process was optimized to improve CMV whole blood recovery using the Alinity m CMV whole blood assay. 465 remnants fresh and frozen CMV whole blood specimens collected at two European institutions (Germany and France) and one US institution were tested using the improved Alinity m CMV whole blood assay. Fresh specimens were stored at 2-8°C while the frozen specimens were stored at -70°C prior to testing. In addition, 37 paired frozen and fresh specimens from the same patients were tested with Alinity m CMV whole blood assay to evaluate the impact of the specimen freeze -thaw on the assay performance.

RESULTS

A total of 465 specimens (420 fresh and 45 frozen) were tested with Alinity m CMV, with an overall validity rate of 99%. 93/465 specimens reported results within the quantitation ranges of Alinity m CMV and RealTime CMV whole blood assays. The mean bias between the two assays (Alinity m CMV minus RealTime CMV) was 0.09 Log IU/mL. In addition, the mean bias between the paired fresh and frozen specimens was -0.06 Log IU/mL (frozen minus fresh).

CONCLUSIONS

Improved Alinity CMV whole blood assay was highly comparable to the RealTime CMV whole blood assay. The Alinity m CMV whole blood assay is performed on the fully automated Alinity m platform which provides short turnaround time testing of urgent samples and same-day reporting of test results thus shortening the time between diagnosis and treatment.

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PERFORMANCE EVALUATION OF ALINITY M EBV WHOLE BLOOD ASSAY

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BACKGROUND-AIM

Quantitation of EBV in whole blood is being used in conjunction with clinical presentation and other laboratory markers as an aid in the diagnosis and management of EBV in transplant and other immunocompromised patients. Alinity m EBV quantitative real-time PCR assay employs a dual-target design to detect highly conserved regions of the EBV genome. The objective of this study was to evaluate the clinical performance of the improved Alinity m EBV whole blood assay when compared to RealTime EBV whole blood assay on the m2000 sp/rt system used in routine clinical testing.

METHODS

Sample preparation process was optimized to improve EBV whole blood recovery using the Alinity m EBV whole blood assay. 374 remnants fresh and frozen EBV whole blood specimens collected at two European institutions and one US institution were tested using the improved Alinity m EBV whole blood assay. Fresh specimens were stored at 2-8°C while the frozen specimens were stored at -70°C prior to testing. In addition, 47 paired frozen and fresh specimens from the same patients were tested with Alinity m EBV whole blood assay to evaluate the impact of the specimen freeze -thaw on the assay performance.

RESULTS

A total of 374 specimens (319 fresh and 55 frozen) were tested with Alinity m EBV, with an overall validity rate of 98.4%. 154/374 specimens reported results within the quantitation ranges of Alinity m EBV and RealTime EBV whole blood assays. The mean bias between the two assays (Alinity m minus RealTime) was -0.14 Log IU/mL. In addition, the mean bias between the paired fresh and frozen specimens was -0.05 Log IU/mL (frozen minus fresh).

CONCLUSIONS

Improved Alinity EBV whole blood assay was highly comparable to the RealTime EBV whole blood assay. The Alinity m EBV whole blood assay is performed on the fully automated Alinity m platform which provides short turnaround time testing of urgent samples and same-day reporting of test results thus shortening the time between diagnosis and treatment.

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PERFORMANCE OF WHOLE BLOOD ASSAY FOR THE QUANTIFICATION OF T-CD4-MEDIATED CMV-SPECIFIC RESPONSE IN HEALTHY SUBJECTS AND IMMUNOCOMPROMISED PATIENTS

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BACKGROUND-AIM

Human Cytomegalovirus (HCMV) is one of the most common viral infections in transplanted patients. HCMV specific T-cell response plays an important role during infection and the role of CD4+IFNg+ have been demonstrated. However, the large majority of whole blood (WB) assays for HCMV T-cell response are mainly restricted to CD8 T-cell response. Thus, the aim of our study was to set-up an "in-house" method for the quantification of pp65-specific CD4+IFNg+ T-cell response in whole blood samples, using recombinant pp65 protein (pp65_rec).

METHODS

Whole blood and peripheral blood mononuclear cells (PBMC) were collected from 50 subjects (25 immunocompetent and 25 immunocompromised). PBMC were used for ELISpot assay (netspots/million PBMC) using both pp65 peptide pool (15 amminoacids with 11 overlap; pp65_pep) and pp65_rec as stimuli. "In-house" WB IFNg release assay (HM-WB IGRA; pg/mL) against the same antigens was performed. The performances of both assays were compared to intracellular staining by flow cytometry (%CD4+IFNg+ and %CD8+IFNg+), as gold standard test.

RESULTS

The cut-off for positive response in ELISpot and HM-WB IGRA was calculated as mean of pp65_ pep and pp65_rec – specific T-cell response of seronegative controls plus two standard deviations.
Overall, using pp65_rec as stimulus in HM-WB IGRA, a good correlation between CD4+IFNg+ was observed, but no correlation was detected with pp65_pep (r=0.5549, p=0.007). On the other hand, no correlation between CD8+IFNg+ and pp65_pep (r=0.4571 p=0.0066) or pp65_rec (r=0.3446, p=0.0459) was detected.
Looking at immunocompetent subjects, a good correlation between pp65_rec and %CD4+ was observed in ELISpot assay (r=0.858, p<0.0001), as well as HM-WB IGRA(r=0.709, p<0.0003). The same trend was observed also in immunocompromised subjects (ELISpot: r=0.82, P=0.0006; HM-WB IGRA r=0.8005, p=0.001). No correlation between two assays and %CD8+ was detected.

CONCLUSIONS

The overall good correlation between "in-house" HM-WB IGRA and %CD4+IFNg+ using the pp65_rec was detected, suggesting that it could be a good method for evaluating CD4+IFNg+response against HCMV. Our results need to be confirmed in a larger sample setting, including both healthy subjects and immunocompromised patients.

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PULMONARY HERPES SIMPLEX VIRUS (HSV) REACTIVATION IN COVID-19 PATIENTS HOSPITALIZED IN ICU

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BACKGROUND-AIM

Herpes simplex virus (HSV) reactivation may occur in critically ill patients with acute respiratory distress syndrome, particularly in COVID patients. Moreover, finding HSV in lower respiratory tract (LRT) from these patients may be associated with an increased mortality. The use of acyclovir (ACV) remains to be determined, since these patients may be at risk of emergence of antivirals resistance. The objectives of this study were to report the rate of HSV reactivation in LRT in COVID patients hospitalized in the intensive care unit (ICU) during the omicron spread and to assess HSV antiviral susceptibility in this context.

METHODS

A monocentric retrospective study was conducted in 3 ICU of the University Hospital of Lyon from January 1st 2022 to December 31th 2022 on ICU patients with COVID diagnosis, in whom LRT samples were addressed to the virology laboratory for HSV testing. HSV detection was performed using HSV1&2 ELITe MGB assay (ELITechGroup, Puteaux, France) and COVID testing was carried with Panther Aptima SARS-CoV-2 assay (Hologic Inc, Marlborough, USA). Antiviral resistance substitutions were detected by NGS (Illumina, San Diego, USA) targeting UL23 and UL30 genes (Giorgi et al. 2023, under submission).

RESULTS

Over the study period, 248 patients underwent COVID and HSV testing from LRT samples: 79 were SARS-CoV-2 positive and 169 were negative. 32 (12.9%) were both SARS-CoV-2 and HSV-positive and 130 (52.4%) were both negative. 39 (15.7%) non-COVID patients experienced HSV reactivation and 47 (19.0%) COVID patients did not. All HSV positive specimens were HSV1 positive. Proportion of HSV reactivation was significantly higher in COVID (40.5%) than in non-COVID patients (23% - p.value < 0.01) with an OR of 2.270 (99% CI 1.075-4.775). Sequencing for antiviral resistance substitutions identified wild-type HSV and HSV harbouring substitutions or indel mutations.

CONCLUSIONS

41% of patients with COVID pneumonia experienced HSV lung reactivation during the period ruled by omicron variant. This result is in accordance with the rate observed during the first COVID wave in spite the lower degree of aggressiveness of this variant, as compared to previous variants. The place of the treatment regarding the risk of emergence of resistance must be further explored.

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QUANTIFERON CMV IN LUNG TRANSPLANTATION: A RETROSPECTIVE STUDY

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BACKGROUND-AIM

Viral replication and Cytomegalovirus (CMV) infection or disease are important complications in lung transplantation. The use of the Quantiferon CMV test (QF-CMV) evaluates the activation of cellular immunity against the virus through the dosage of Interferon ©, released by activated CD8+ T lymphocytes and is required in the monitoring of patients undergoing lung transplantation in order to of predict CMV infection in these subjects. The aim of the study is to identify in the Quantiferon CMV test a predictive tool on the clinical progress of the lung transplant patient, in particular in the development of rejection.

METHODS

The study was conducted at the University Hospital Città della Salute e della Scienza of Turin, Microbiology and Virology Unit and Pneumology Unit. Viral replication on bronchoalveolar lavage (BAL) and blood (CMV ELITe MGB Kit – ELITechGroup) and the activation of cellular immunity against CMV (QuantiFERON-CMV, QIAGEN) were evaluated in 63 patients undergoing lung transplantation. The patients were divided into three groups: group A-Donor/Recipient CMV negative, group B-Recipient CMV positive and with non-reactive QF-CMV, group C-Recipient CMV positive and with reactive QF-CMV.

RESULTS

Positive CMV DNA results on BAL were consistently higher in groups B and C than in group A, while no significant differences were observed on blood. Percentage of acute rejections were higher in group B than in the other groups. Two years after transplantation, more patients diagnosed with chronic rejection were found in group B than in group C (p 0.0164).

CONCLUSIONS

The study evaluated the response to CMV in lung transplant patients and allowed us to affirm the validity of the prophylaxis therapy for the virus used in our centre; with the execution of the Quantiferon CMV test we observed how seropositivity for CMV in the absence of reactivity for QF-CMV exposes patients to the onset of complications and adverse outcomes such as acute and chronic rejections.

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RECOMBINANT ZOSTER VACCINE REVEALED ROBUST HUMORAL AND CELL-MEDIATED IMMUNOGENICITY IN PATIENTS WITH SOLID TUMOURS

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BACKGROUND-AIM

Herpes zoster (HZ) is caused by reactivation of latent varicella zoster virus (VZV). Severely immunocompromising conditions, such as solid tumours, have been largely associated with an increased risk for HZ due to diminished VZV-specific cellular immunity. Recombinant zoster vaccine (RZV) might prevent severe HZ complications changing perspectives in immunocompromised subjects. The aim of this study was to evaluate the humoral and cell-mediated immunity in solid tumour patients before and 3 weeks after complete RZV vaccination schedule.

METHODS

38 patients (median age 71 years, 73% male) were enrolled between November and December 2022. Sera and peripheral blood mononuclear cells (PBMCs) were collected at baseline (T0) and at 3 weeks after complete vaccination schedule (T2). IFN-© ELISpot assay was performed against gE and IE63 peptide pools. In parallel, antigen-specific lymphocytes were identified by the expression of IFN-© using flow cytometry. VZV-specific antibodies were quantified at each time point.

RESULTS

At enrollment 37/38 participants (99%) were VZV-seropositive. Among RZV vaccine recipients found an increase of VZV-IgG level of at least 2-fold at 3 weeks after complete vaccination in 25/38 (65%) subjects. T-cell mediated response was detected in 35 enrolled patients. At baseline, only 16/35 (45.7%) subjects were positive for gE-specific T-cell response while the frequency of “responders” patients reached 30/35 (85.7%) after complete vaccination schedule. The median level of response increased from 10 [IQR 1-40] net spots/million PBMC to 45 [IQR 15-125] net spots/million PBMC ($p<0.0001$). An increase of response of at least 2-fold was considered for definition of “responder patients” and observed in 23/35 patients (65.7%). On the other hand, no increase was observed against IE63 peptide pool ($p=0.618$). When measured by flow cytometry assay the overall IFN©-producing lymphocytes increase was mainly associated with CD4 and CD8 T-cell response ($p<0.0001$, $p=0.006$ respectively).

CONCLUSIONS

The “real-life” data on RZV administration in a cohort of solid tumor patients revealed a high immunogenicity of the vaccine with more than 65% of responder patients. Efficacy should be evaluated in prospective long-term studies.

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ROLE OF EPSTEIN-BARR (EBV)-SPECIFIC T-CELL RESPONSE MONITORING IN KIDNEY TRANSPLANT RECIPIENTS (KTRS)

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BACKGROUND-AIM

Epstein-Barr virus (EBV) still represents a great concern in solid organ transplant recipients. The role of EBV-specific T-cell response in prediction of EBV severe infection need to be assessed. In this study, we aimed to analyze the kinetics of T cell-mediated response against EBV antigens in a cohort of Kidney transplant patients (KTRs).

METHODS

Sixty-two subjects were enrolled at time of transplant and EBV DNA was monitored according to diagnostic procedures. From 32 patients, peripheral blood mononuclear cell (PBMC) were collected at time of transplant and at 60, 120, 240 and 360 days and used for quantification of IFN-© response by ELIspot assay. The IFN-© response was evaluated against three peptides pools of EBV (EBNA, LMP and lytic) and results were given as IFN-© spot forming units (SFU)/million PBMC. T- CD4+ cells/ʃl were determined by flow cytometry. Demographic and clinical data have been also collected.

RESULTS

EBV DNA was detected in 26/62 (42%) patients and the peak of EBV DNAemia was measured at median day after transplant of 48 days (IQR 20-154). EBV-specific T cell response against three groups of antigens at different time points was measured. T-cell response against lytic pool measured at 60 days after transplant was significantly lower than that detected at the end of follow up (p=0.0106), even if no differences were observed when EBNA and LMP pool were measured. According with T-CD3CD4+ count measured at 60 days, patients were classified in two groups, i) <200 and ii) >200 cell/ʃl. Overall, 8/32 (25%) subjects showed T-CD4+ count <200 cell/ʃl and, of them, 6/8 (75%) showed at least one episode of EBV infection. On the other hand, 24/32 showed T-CD4+ > 200 cell/ʃl and only 7/24 (29%) showed at least one episode of EBV infection (p=0.0383). Looking at T cell response against lytic pool, a significant difference was observed at 240 days between the two group of patients (p=0.0372) .

CONCLUSIONS

A differential kinetics of EBV lytic pool T cell response was observed in our study. Despite the low number of patients, the data suggest a role of EBV-specific T-cell response and T-cell count in EBV DNA occurrence. Further studies are needed to understand the potential role of EBV-specific immune monitoring in KTRs.

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SCREENING FOR HTLV 1/2 IN DECEASED DONORS- 10 YEARS COMPLETE NATIONAL DATA FROM A LOW PREVALENCE COUNTRY

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BACKGROUND-AIM

Human T-cell lymphotropic virus-1(HTLV-1) is an oncogenic retrovirus and the etiological agent for adult T-cell leukemia(ATL) and HTLV-associated myelopathy/tropical spastic paraparesis(HAM/TSP).The prevalence of HTLV-1 is low in Europe.
The transmission of HTLV-1 occurs through sexual contact, mother to child transmission, via blood transfusion, or organ transplantation. The risk of HAM/TSP is highest in seronegative recipients transplanted with organs from seropositive donors.
Screening of solid organ donors for HTLV-1 is currently not recommended in Europe due to the low prevalence and the risk that false positive results should cause wastage of organs. We wanted to evaluate the occurrence of reactive HTLV-samples among deceased donors in Norway and its consequences for transplantation.

METHODS

All solid organ transplant activity in Norway is located at the national transplant centre at Oslo University Hospital, Rikshospitalet, and all blood specimens collected from eligible deceased donors are analysed at the Department of Microbiology.
In 2012 routine screening for HTLV-1/2 antibodies was established. All samples were tested with the Abbot Architect HTLV- I/II assay. Reactive samples were retested in duplicate, and if still reactive, analysed with an alternative test, MP Diagnostics (HTLV-1/2 Elisa 4.0). If the alternative assay was not negative, immunoblot was performed.

RESULTS

From 2012 to 2023, 2694 samples from deceased donors have been analysed. In the primary test 2671(99%) were negative, 5(0.2%) were grey-zone reactive, 5(0.2%) were weakly positive, and 13(0.5%) were positive.
Among the reactive 23 samples, 6 of the positive tests were also reactive in the secondary test and were sent for immunoblot analysis. The immunoblot was negative in 3, inconclusive in 2, and not performed in 1 of the donor sera.
Organs were used from 5 of the 23 reactive donors (3 weakly positive, 2 positive in the primary test).

CONCLUSIONS

In complete national deceased donor data from a 10 year period, 1% of the samples screened were HTLV-reactive. After confirmatory testing, only 3 of these 23(0.11%) donors could not be confirmed to be negative. With universal screening of deceased donors in a low-prevalence area caution must be taken to avoid unnecessary organ wastage.

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SEROPREVALENCE OF CYTOMEGALOVIRUS OVER THE LAST 2 DECADES (2000 – 2020), A RETROSPECTIVE DATA ANALYSIS FROM A SINGLE LABORATORY

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BACKGROUND-AIM

CMV is ubiquitous however, seroprevalence varies geographically across the world in different age groups. The aim of this study is to retrospectively analyse trend data on CMV seroprevalence over the last 2 decades by gender, age and ethnicity obtained from a single laboratory, serving 4 hospitals in SingHealth for this assay.

METHODS

We analysed laboratory data from Singapore citizens and permanent residents with CMV IgG results from 2001 to 2020.
The associations of seroprevalence with age, gender, ethnic group and year were analysed using multivariate logistic regression analysis. Age and year were categorized into ordinal variables:
• The study was stratified into two time periods: Period 1 (2001-2010) and Period 2 (2011-2020).
• Age was categorized into six age groups: 2-14, 15-24, 25-34, 35-44, 45-54, and ε 55 years.
• Ethnicity was grouped into Chinese, Malays, Indians, and Others.
Fisher's exact test was used to compare the categorical variables and multiple comparisons were corrected using the FDR_{B-H} method.

RESULTS

1. There was a declining trend of seropositivity over the years, from a peak of 87.9% in 2002 to 73.7% in 2020.
2. Seroprevalence increased with age but showed significant differences between Period 1 and Period 2.
3. Seropositivity decreased significantly in Period 2 for both genders in the age groups of ε 25 years, with the largest decline observed in the age group 25-34 years for males and age group 35-44 years in females.
4. Between the 2 time periods, seropositivity for those in the Chinese ethnic group decreased significantly in age groups of ε 25 years, mirroring the overall trend of CMV seroprevalence. The other 2 ethnic groups also experienced a decline in seropositivity across most age groups in Period 2.

CONCLUSIONS

A decline in CMV seroprevalence is observed in our study group and is strongly associated with age, gender, and ethnicity. Because infection and reactivation of latent CMV may contribute to morbidity and mortality in immunocompromised patients and affect CMV-naive pregnancies, understanding the seroprevalence may advise CMV preventive strategies and guide future vaccine development efforts.

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THE EFFECT OF TACROLIMUS AND MYCOPHENOLIC ACID LEVELS ON TTV LOAD IN KIDNEY TRANSPLANT RECIPIENTS

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BACKGROUND-AIM

Load monitoring of torque teno virus (TTV), a non-pathogenic commensal virus, has been proposed as an indicator to signal both insufficient and excessive immunosuppression after solid organ transplantation. As such, the actual TTV-load could guide immunosuppressant dosing and prevent complications like organ rejection and infection, respectively. However, the relation between TTV-load kinetics and immunosuppressant pharmacokinetics has not been studied in detail. This study aimed to systematically investigate the effect of exposure to commonly used immunosuppressants, tacrolimus (Tac) and mycophenolic acid (MPA/MMF), on TTV-load in kidney transplant recipients.

METHODS

A database was designed that contained extensive therapeutic drug and TTV-load monitoring measurements of a cohort of 255 kidney transplant recipients (KTR) transplanted between 2005-2012 on immunosuppressive maintenance therapy with Tac, MPA and prednisolone. We explored the effect of Tac trough level (C0), Tac area-under-the-curve concentration (AUC0-12h) and MPA AUC0-12h on the TTV-load measured by quantitative PCR at months 1.5, 3, 6 and 12, and on changes in the TTV-load between months 1.5-3, 3-6 and 6-12. Multivariable linear regression models were performed to assess coefficients adjusted for confounders.

RESULTS

Measured TTV-loads peaked at month 3 posttransplantation and lowered thereafter. Per µg/L Tac C0 increase, a slight increase in TTV load was observed at months 3, 6 and 12, which was significant at months 3 and 12 (M3 ® 0.12 [CI 0.03;0.22]; M6: 0.07 [CI -0.04;0.17]; M12: 0.17 [CI 0.07;0.28]). Results were comparable for Tac AUC0-12h measurements. No significant effect was found for MPA, although the effect did increase over time. For both drugs, no effect was found on the change in TTV load.

CONCLUSIONS

A modest effect of Tac exposure on TTV-load was found in the first year after transplantation. The large variation in TTV-loads observed among recipients after kidney transplantation, therefore, is not fully explained by exposure to the Tac and MPA immunosuppressants, and further study is needed to understand the relationship between TTV-load, immunity and immunosuppression.

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THE ROLE OF MIR-144 IN THE PROLIFERATION AND MIGRATION OF HUMAN CYTOMEGALOVIRUS (HCMV) INFECTED AND NON-INFECTED GLIOBLASTOMA CELLS

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BACKGROUND-AIM

Glioblastoma (GBM) is one of the most common and deadly malignant brain tumors, remaining an incurable disease with very poor prognosis even to this day. Human cytomegalovirus (HCMV) and glioblastoma are often closely associated, as HCMV is proposed to play an oncomodulatory role in glioblastoma, promoting the malignant properties of cells, such as proliferation and migration. miR-144 is an important regulator of oncogenic processes and has been found to be down-regulated in glioblastoma tissue samples. Taking into consideration the previously mentioned facts, this study aims to investigate the possible role of miR-144 in regulating the proliferation and migration of mock and HCMV infected U373MG glioblastoma cells.

METHODS

To explore miR-144 function in glioblastoma, mock and HCMV infected U373MG cells were transfected with miR-144 mimics or miR-144 inhibitors and the efficiency of transfection was validated via qRT-PCR. MTT and wound healing assays were used to measure cell proliferation and migration respectively, and immunofluorescence experiments were performed to further study cell morphology.

RESULTS

We found that over-expression of miR-144 significantly suppressed cell growth in both HCMV infected and non-infected cells, while miR-144 inhibition allowed cells to maintain their standard proliferative capacity. Similarly, increased expression of miR-144 led to inhibition of cell migration, especially in mock cells compared to infected cells. Both infected and non-infected cells with decreased miR-144 expression exhibited the same migration rate as control cells. In addition, U373MG cells over-expressing miR-144 appeared to display altered cell shape and cytoskeleton organization compared to control cells.

CONCLUSIONS

These observations indicate that miR-144 over-expression induces alterations in the proliferation and migration rate of both mock and HCMV infected U373MG cells. This suppressive effect might be caused by the reformation of cell shape in miR-144 over-expressing cells and might come out as a beneficial therapeutic target for treatment in the future.

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